DIFFERENTIATION BETWEEN VITAMIN G AND AN INSOLUBLE FACTOR PREVENTING A PELLAGRA-LIKE SYNDROME IN CHICKS [1]

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ONE TEXT FIGURE AND ONE PLATE

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In studies designed to throw further light on the cause of a peculiar paralysis of nutritive origin (Norris et al., '31) involving the legs and feet of chicks, Ringrose, Norris and Heuser ('31) observed a pellagra-like syndrome for the first time in this species. This pellagra-like syndrome was comparable to the pellagra in the albino rat obtained by Goldberger and Lillie ('26) and Salmon, Hays and Guerrant ('28) in so far at least as one could expect to find identical symptoms in so widely different species.

In this experimental work diets containing commercial casein, purified casein or dried raw egg white together with cereals, cod liver oil, and necessary minerals were fed. Three comparable experiments were conducted. In the first and second experiments the same supply of ingredients was used but in the third experiment a different supply was used.

In all experiments external lesions of a distinctly pellagrous character appeared in the lots receiving the egg-white diet at about 3 weeks of age. A little later the lots receiving the

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commercial-casein diet and those receiving the purified-casein diet also developed pellagrous lesions identical in appearance in so far as could be determined by detailed macroscopic examination to those which developed in the chicks on the egg-white diet. Pictures showing the striking similarity of the pellagra-like syndrome on the casein diets and the egg-white diet are presented in plate 1.

In this syndrome lesions developed at the eyes, the corners of the mouth and on the feet. The margins of the eyelids became granular and a viscous exudate was produced which frequently caused the eyelids to stick together so that the chicks could not see. Crusty scabs appeared at the corners of the mouth. These scabs gradually enlarged to involve the margins of the skin around the nostrils and underneath the lower mandible. The skin on the bottoms of the feet and between the toes gradually thickened and cornified, and small cracks and fissures appeared. These enlarged and deepened and occasionally slight hemorrhages occurred. Usually the severely affected chicks were sensitive to walking.

The average intensity of the pellagra-like syndrome of the lots of chicks on the casein diets was never as severe as that of the lots of chicks on the egg-white diet, although there were individuals on the casein diets which were just as severely affected as those on the egg-white diet.

The mortality of the chicks receiving the egg-white diet was very high in all cases, the average being 81.8% at the end of the 12-week experimental period as compared to an average of 48.7% in the chicks receiving the purified-casein diet.

The chicks in the lots receiving the egg-white diet grew at a more rapid rate at the start, than did the chicks in the lots receiving the purified-casein diet. Later, however, at the onset of the pellagra-like syndrome the growth of the chicks on the egg-white diet was slowed up sufficiently so that the growth of the chicks on the purified-casein diet finally equaled or exceeded that of the chicks on the egg-white diet. In figure 1 are presented the growth curves of these lots and also the growth curves of comparable lots used as controls in two

subsequent experiments. However, in one of these the growth of the chicks fed the purified-casein diet never quite equaled that of the chicks fed the egg-white diet.

The biometrical significance of the mean growth difference at 3 weeks of age, the approximate age of the greatest difference, between the average of the mean weights of the lots fed the purified-casein diet and the lots fed the egg-white diet in all experiments was 13.5 times greater than the standard error of the difference.[3] This difference in growth was, therefore, highly significant.

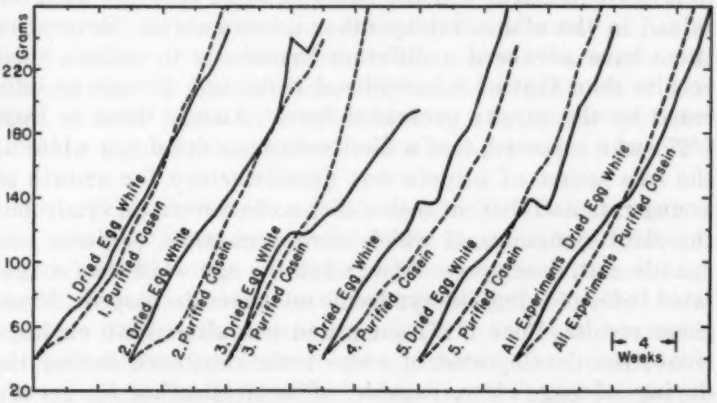

Fig. 1 Difference in growth response between the chicks fed the egg-white diet and those fed the purified-casein diet.

It was evident, then, that the egg-white diet was greatly deficient in a pellagra-preventing factor but contained a fairly large amount of a growth-promoting factor while the purified-casein diet was nearly adequate in a pellagra-preventing factor but very deficient in a growth-promoting factor. In view of these results it was felt desirable to make a further study of the possibility of the existence of a factor required to prevent the occurrence of the pellagra-like syndrome on the egg-white diet and of a factor required to promote normal growth on

[3] Under the conditions of these experiments a difference 2.5 times greater than its standard error is significant.

the purified-casein diet. The growth-promoting factor was vitamin G (B_2) in the sense of the term generally accepted at the time this investigation was undertaken, since Ringrose, Norris and Heuser ('31) found that autoclaved yeast promoted growth and prevented the development of pellagra-like lesions in chicks, fed a cereal, casein diet deficient in this vitamin. Whether or not the pellagra-preventing factor had been considered a part of a so-called vitamin G complex up to this time could not be determined from this evidence.

A syndrome similar to the pellagra-like syndrome which developed in chicks fed the raw egg-white diet has been obtained in the albino rat by other investigators. Several of these have advanced a different hypothesis to explain their results than that of a nutritional deficiency disease as indicated by the results presented here. Among these is Boas ('27) who reported, that a diet containing dried egg white as the sole source of protein was unsatisfactory for growth in young rats and that on such a diet a characteristic syndrome, the chief symptoms of which were dermatitis, baldness and spastic gait, developed. Also, when the egg white was coagulated before feeding the syndrome no longer developed. From these results, Boas ('27) suggested two alternative explanations; the development of some toxic substance during the drying of egg white, capable of neutralization by certain protective foods or the destruction of some hitherto unrecognized dietary essential when drying the egg white before coagulation. However, neither explanation was entirely satisfactory.

Findlay and Stern ('29) confirmed the finding of Boas that dried egg white resulted in nervousness and cutaneous symptoms but their investigations of the pathological changes occurring led them to believe that the condition in rats was similar to Swift's disease in children. The fact that the same disease was produced in suckling rats whose mothers received dried egg white caused them to postulate the deficiency of a dietetic factor as opposed to the presence of a toxic substance.

Boas-Fixsen ('31) after further work came to the conclusion, however, that the weight of evidence favored the explanation that dried egg white contained some toxic substance created during the drying process and opposed the theory that the nutritive disorder was due to a deficiency as believed by Findlay and Stern.

Parsons ('31) pointed out that, of the diets rich in protein studied, the one high in egg white was the only one exhibiting markedly unfavorable physiological effects on rats in striking contrast to results obtained with diets high in casein, dried liver, beef powder and egg yolk. She favored the theory of a toxic principle rendered innocuous by the presence of a protective factor or by cooking of the egg white. On rations containing 66% of dried or fresh egg white, supplemented with 20% of dried yeast, 20% of dried liver was required to prevent the development or to cure the characteristic symptoms in rats.

Boas-Fixsen and Parsons both recognized that the symptoms which they obtained on egg-white diets were similar to pellagra but did not feel that a deficiency theory or lack of a dietary essential such as vitamin G satisfactorily explained their results. Their adherence to the toxic theory was prompted primarily by the fact that cooked egg white no longer exhibited abnormal physiological effects. Moreover, Parsons reported a decrease in rate of growth with increase in quantity of egg white in the diet and used this evidence in support of the toxic theory. Boas found that a reduction of dried egg white to one-half the usual amount did not decrease the severity of the symptoms produced.

In spite of the reports of Boas-Fixsen and of Parsons it did not seem reasonable that egg white contained a toxic factor which could be destroyed by cooking. It seemed more probable that the egg-white diet was lacking in some dietary essential which was supplied by other food substances as held by Findlay and Stern in view of the apparent identity of the pellagra-like syndrome which developed on the egg-white and purified-casein diets used in this investigation.

The fact that egg white is almost the only source of protein used by the developing chick embryo (Needham, '31) not only gives support to this hypothesis but also suggests a more probable explanation for the failure of the pellagra-like syndrome to develop on cooked egg white than the toxicity theory of Boas-Fixsen and of Parsons. This is that, during the coagulation of egg white, there is formed or released from egg white a factor which prevents the pellagra-like syndrome and that the pellagra-preventing factor cannot be formed or released from raw egg white by the normal digestive processes of the animal or chick whereas in the normal metabolic processes of the developing chick embryo this occurs.

EXPERIMENTAL PROCEDURE

In these studies single comb White Leghorn chicks of the Cornell strain were used. In general the lots contained twenty chicks each. The chicks in each lot were individually weighed, identified by wing bands and placed in the experimental pens approximately 24 hours after hatching. Thereafter they were weighed individually every week for the duration of the 8-week experimental period. Brooders and pens were equipped with screen floors in order to prevent coprophagy. Feed and water were kept continuously before the chicks throughout the duration of each experiment. A weekly record of feed consumption was kept.

The diets were so composed as to supply protein at a level of 25% of the dry matter. This corresponds to approximately 22% air dry basis. Such a level of protein is greater than that found by this laboratory (Norris and Heuser, '30) to be required to supply a sufficient amount for normal growth. Thus, the possibility of the quantity of protein becoming a limiting factor in the experimental diets was removed.

In order to determine the severity of the pellagra-like syndrome which developed on the various diets, weekly observations of each chick were made at the time of weighing. These observations were made by the same observer and were based on a consideration of the lesions at the corners of the

mouth, at the eyes and on the feet. These, when present, were recorded by a numerical rating of 1, 2 or 3, meaning respectively slight, medium or severe. The lesions at the mouth were designated M_1, M_2 or M_3; at the eyes E_1, E_2 or E_3 and on the feet F_1, F_2 or F_3. This gave a possible maximum degree of severity of 9 when the three symptoms were each rated as 3. The total degree of severity of the pellagra-like syndrome for any week was determined by adding up the sub-numbers for all the afflicted chicks in a group and dividing by the total number of chicks in that group times 9. This figure represented the maximum severity possible. When multiplied by 100, an expression of the percentage degree of severity of the pellagra-like syndrome for any group of chicks for any week was obtained. The necessary calculations are represented by the following formula:

$$\frac{M_n + E_n + F_n}{C \times 9} \times 100 = \% \text{ degree of pellagra-like syndrome}$$

Where n = observed severity of pellagrous lesion at M (mouth), at E (eyes) and F (feet). C = number of chicks per lot.

Preliminary experiments were undertaken for the purpose of finding suitable supplements to the egg-white diet which would prevent the development of the pellagra-like syndrome and give normal growth. A typical control diet containing egg white is given below:

	%
Yellow corn meal	58.75
Wheat flour middlings	20.00
Egg white	16.75
Cod liver oil	1.00
Steamed bone meal	2.00
Limestone	1.00
Salt (iodized)	0.50
	100.00

The dried egg white[4] used in this work was a commercial dried undenatured product which was finely ground. It received no special treatment before being incorporated into the diet.

[4] Obtained from Jaburgh Bros., New York City.

The results of these experiments showed that dried yeast, autoclaved yeast or dried egg yolk fed at a 10% level in the egg-white diet did not prevent the syndrome. However, the feeding of 10% of dried liver, prepared from frozen pigs' liver by moderate cooking, gave normal growth and entirely prevented the pellagra-like syndrome throughout the experimental period of 8 weeks. In a subsequent experiment it was found that 7.5% of dried liver was sufficient for this purpose. Therefore, dried pork liver was used at the 7.5% level in further studies on this problem.

The first experiment was undertaken in order to find, if possible, a method or methods whereby the factor in liver which prevents the pellagra-like syndrome could be destroyed. Four different treatments were applied to the liver as follows: Wet autoclaving for 6 hours and 30 hours, alkaline autoclaving for 6 hours and dry autoclaving for 30 hours. In the preparation of wet-autoclaved liver, dried liver meal was mixed with water and allowed to soak until the whole was a jelly-like mass. This mixture was then placed in the autoclave in large pans 2 inches deep. The pressure in the autoclave was maintained at 15 pounds for 6 hours when part of the liver was removed, the remainder being autoclaved at 15 pounds until 30 hours had elapsed. The two lots, autoclaved wet, were then dried in a stream of air and ground. The alkaline autoclaved liver was prepared by mixing dried liver with water, until it could be readily stirred with a wooden paddle when a sodium hydroxide solution (approximately 25%) was added until pH 9 was reached as determined by the hydrogen electrode. The alkalized liver was allowed to stand over night during which time practically no reduction in pH occurred. Then it was placed in pans in the autoclave and autoclaved for 6 hours at 15 pounds' pressure. During the autoclaving the pH dropped to about 7.5. The material was then brought to pH 4.5 to 5.0, dried in a stream of air and ground. Dry-autoclaved liver was prepared by placing dried liver without previous addition of water in pans in the autoclave and autoclaving for 30 hours at 15 pounds pressure. This material absorbed so little

water during this process that subsequent drying and grinding was unnecessary.

These different liver preparations were then fed at a level of 7.5% by including them in the egg-white diet in such a way as to maintain the protein level at 25% dry basis. The results are presented in table 1. The control egg-white diet gave the usual results in the way of poor growth, high mortality and severe pellagra-like lesions. The egg-white diet containing 7.5% dried liver which served as the positive control gave normal growth with no mortality and no pellagra-like lesions.

TABLE 1

Treatment of liver to destroy its pellagra-preventing properties when combined with the egg-white diet

EXPERIMENT NO.	LOT	GROWTH, WEEKS 8	MORTALITY, WEEKS 8	PELLAGRA, WEEKS		
				4	6	8
		gm.	%	%	%	%
8	EW (egg white)	268	35	57	56	55
8	EW + 7.5% liver	666	..	..	..	..
8	EW + 7.5% liver A[1] wet 6 hours	568	..	..	..	..
8	EW + 7.5% liver A pH 9 6 hours	577	..	..	..	..
8	EW + 7.5% liver A wet 30 hours	417	5	..	..	..
8	EW + 7.5% liver A dry 30 hours	296	15	23	33	33
7	EW + 7.5% liver A dry 30 hours	362	32	60	52	28

[1] A = autoclaved.

The results from the lots fed egg-white diets containing liver wet autoclaved for 6 and 30 hours showed that neither of these treatments destroyed the pellagra-preventing factor in liver. However, the growth was somewhat less than that on the untreated liver, particularly on the liver which was autoclaved wet for 30 hours.

The alkaline autoclaving at pH 9 also did not destroy the pellagra-preventing factor of liver since no pellagric lesions occurred. Again the growth response in this lot was somewhat below that of the lot on the untreated liver.

The results obtained by feeding liver dry autoclaved for 30 hours showed that this treatment destroyed the pellagra-preventing factor in liver so completely that the pellagra-like

syndrome was as severe as on the control diet. The results confirmed those of a preliminary experiment in which there was one lot fed the egg-white diet containing liver dry autoclaved for 30 hours. These results are presented in table 1 for comparison with those of the present experiment. The pellagra-preventing factor therefore was unstable to dry autoclaving. These results on dry autoclaving together with the appearance of a paper by Kline et al. ('32) in which it was reported that dry heat treatment destroyed a pellagra-preventing factor required by chicks led to the adoption of the latter treatment in the next experiment.

This experiment was undertaken to obtain a separation of the factor which prevented the pellagra-like syndrome from the growth-promoting factor. In this experiment the egg-white diet was used to determine the presence of the pellagra-preventing factor and the purified-casein diet was used to determine the presence of the growth-promoting factor. It has already been pointed out that the egg-white diet was well supplied with the growth-promoting factor and that the purified-casein diet was low in this factor. The purified-casein diet used in this experiment is given below:

	%
Yellow corn meal	61.50
Wheat flour middlings	20.00
Purified casein	14.00
Cod liver oil	1.00
Steamed bone meal	1.00
Limestone	2.00
Salt (iodized)	0.50
	100.00

Dried liver was used as a source of both the growth-promoting and pellagra-preventing factors. Three different treatments were given this material in attempting to separate the factors. These were heating in a dry atmosphere, alkaline autoclaving and extraction.

The heated liver was prepared by heating the dried liver in thin layers in a dry atmosphere for 50 hours at 120°C. The autoclaved liver was prepared by treating the dried liver

with a sodium hydroxide solution with stirring until the pH was 11 as determined with a hydrogen electrode. The mixture was then allowed to stand overnight and, if the pH had fallen, (never fell below pH 10.5) more alkali was added to bring it back to pH 11 before placing in the autoclave. After the material had been autoclaved at 15 pounds pressure for 6 hours it was removed from the autoclave and a pH determination made. It was found that the pH dropped during the autoclaving to pH 9.4. Sufficient hydrochloric acid was then added to bring the pH to 4.5 to 5.0, when the liquid mass was dried and ground.

An extract of dried liver was prepared by using an ethyl alcohol-water mixture containing 20% alcohol by weight. The liver was first defatted with ether, then air-dried and the equivalent of 1 kilo. dried liver (approximately 850 gm. defatted liver) was treated with 2 liters of the alcohol-water mixture, allowed to stand for an hour with frequent stirring and then filtered. The filtrate was then brought to a temperature of 80°C. to coagulate any protein remaining and filtered a second time. This extraction was repeated twelve times, the filtrate becoming practically colorless at the end of that time. The combined filtrates (approximately 24 liters) were concentrated under a vacuum of 650 mm. of Hg and the volume adjusted so that 1 cc. was equivalent to 1 gm. of the original dried liver. After extraction the residue was dried and ground.

This experiment contained twelve lots of chicks, six of which received the egg-white diet while the other six received the purified-casein diet. Dried liver, liver extract, liver residue, heated liver and alkaline autoclaved liver were used as supplements for both diets. The lots on the egg-white diet received the liver supplements at a level equivalent to 7.5%. Since preliminary results had shown that 2.5% of dried liver promoted normal growth in chicks fed the purified-casein diet the lots on this diet received the liver supplements at a level equivalent to 2.5% of dried liver.

The summary of the results of this experiment are presented in table 2 together with those of a duplicate experiment in which a different supply of egg white, purified casein and dried liver were used. The various supplements were prepared in the same way and fed at the same levels as in the original experiment.

The chicks on the egg-white control diet responded as usual with more rapid growth at the start than those on the purified-casein control diet but slowed up at the onset of the syndrome

TABLE 2

Differentiation of the growth promoting factor from the pellagra-preventing factor

EXPERIMENT NO.	LOT	GROWTH, WEEKS 8	MORTALITY, WEEKS 8	PELLAGRA, WEEKS		
				4	6	8
		gm.	%	%	%	%
13	EW (egg white)	206	30	41	56	53
14	EW (egg white)	227	45	61	67	64
13	EW + 7.5% liver	648	5	..	..	..
14	EW + 7.5% liver	646	15	..	..	..
13	EW + liver extract [1]	286	40	35	68	55
14	EW + liver extract [1]	219	70	62	80	78
13	EW + liver residue [2]	578	5	3	..	..
14	EW + liver residue [2]	604	15	13	8	..
13	EW + heated liver [2, 3]	356	55	21	37	44
14	EW + heated liver [2, 3]	363	55	52	51	47
13	EW + A liver [2, 4]	342	30	22	32	48
14	EW + A liver [2, 4]	347	20	47	50	48
13	PC (purified casein)	242	40	..	..	..
14	PC (purified casein)	357	15	..	..	..
13	PC + 2.5% liver	703	..	..	..	..
14	PC + 2.5% liver	652	10	..	..	..
13	PC + liver extract [2]	567	..	..	..	..
14	PC + liver extract [2]	621	10	..	..	..
13	PC + liver residue [2]	402	5	..	..	..
14	PC + liver residue [2]	485	10	..	..	..
13	PC + heated liver [2, 3]	586	10	..	..	..
14	PC + heated liver [2, 3]	665	10	..	..	..
13	PC + A liver [2, 4]	388	10	..	..	..
14	PC + A liver [2, 4]	631	10	..	..	..

[1] Equivalent to 7.5% liver.

[2] Equivalent to 2.5% liver.

[3] Liver heated for 50 hours at 120°C.

[4] Liver autoclaved at pH 11 for 6 hours at 15 pounds.

so that the growth of the purified-casein chicks finally exceeded that of the egg-white chicks. Severe pellagra-like lesions developed in the chicks on the egg-white diet causing a higher average mortality than that occurring in the chicks on the purified-casein diet in which none of the syndrome developed.

The addition of dried liver to the egg-white diet caused a normal rate of growth to take place and the development of the syndrome was prevented. The addition of dried liver to the purified-casein diet also caused normal growth.

Whenever liver extract displaced dried liver in the egg-white diet the pellagra-like syndrome was severe and a high mortality occurred. Growth was just as poor as on the control diet. The lots receiving the extracted residue, however, showed practically no symptoms of the syndrome and little mortality. Growth was nearly equal to that of the unextracted liver. On the other hand, whenever liver extract displaced dried liver in the purified-casein diet, practically normal growth resulted whereas the liver residue gave growth only slightly better than the purified-casein control. The mortality was small in both cases.

The chicks of the two lots fed the egg-white diet containing liver heated in a dry atmosphere for 50 hours at 120°C. developed severe pellagra-like lesions, the mortality was high and growth was no better than that of the control lots. This showed that dry heat treatment destroyed practically all of the pellagra-preventing factor of liver. These results are in agreement with those presented in table 1 which showed that liver autoclaved dry for 30 hours also destroyed the pellagra-preventing factor. In striking contrast to these results the chicks of the two lots fed the purified-casein diet containing liver heated in a dry atmosphere showed that this liver induced a rapid rate of growth with no mortality and none of the pellagra-like syndrome.

Alkaline autoclaving at pH 11 also destroyed the pellagra-preventing factor of liver as shown by the results from those lots which received the egg-white diet containing alkaline

autoclaved liver. The pellagra-like syndrome was practically as severe, the mortality as high and the growth as poor as on the egg-white control lots or those receiving heated liver or liver extract. In the first experiment on the purified-casein diet the results of alkaline autoclaving liver at pH 11 showed that the growth-promoting factor of liver was also sensitive to this treatment, but in the second experiment little destruction of this factor took place. In both cases the liver was at pH 11.0 to 11.3 when put into the autoclave and was at pH 9.4 when removed. However, a different supply of dried liver was used in each experiment. Possibly then the growth-promoting factor was afforded greater protection by one supply of liver than by the other.

The results of these duplicate experiments show, therefore, that the factor which prevented the pellagra-like syndrome on the egg-white diet is different from the one which promoted growth on the purified-casein diet, since the pellagra-preventing factor was insoluble in the alcohol-water mixture, was destroyed by heating in a dry atmosphere and was somewhat less sensitive to alkaline autoclaving at pH 11.

These results are in accord with the findings of Boas-Fixsen ('31) that the factor which prevents the pellagra-like syndrome is insoluble in water. She showed that casein extracted with dilute acetic acid solution and yeast extracted with boiling dilute acetic acid solution still contained the protective factor.

The results of Parsons and Lease ('34) published after the completion of the work presented in this report showed that the factor in liver which prevents or cures the pellagra-like syndrome in rats developed by feeding egg-white diets is contained in the solid liver residue remaining from the preparation of Eli Lilly and Company liver extract no. 343 and is not present in the extract and that it is destroyed by dry heating liver for 6 days at 100°C. Later Lease and Parsons ('34) in work with chicks fed an egg-white diet offered further confirmation of the fact that the factor concerned is not removed from liver by extraction but remains in the extracted residue.

Salmon and Goodman ('34) showed likewise that well-developed cases of the syndrome in rats were apparently cured by brewer's yeast and the extracted residue of brewer's yeast but not by the yeast extract or by extracted casein. The casein may have been fed, however, at too low levels, since Gorter ('35) has shown that purified casein contains the factor required to prevent the egg-white syndrome in rats.

In experiments on the stability of the pellagra-preventing factor, not reported here, studies of the effect of autoclaving egg white were made. The autoclaving was carried out by placing dried egg white at its natural pH (5.9 to 6.0) in pans in the autoclave at a pressure of 15 pounds for 6 hours, after

TABLE 3

Effect of autoclaving on the pellagra-developing characteristics of egg white

EXPERIMENT NO.	LOT	GROWTH, WEEKS 8	MORTALITY, WEEKS 8	PELLAGRA, WEEKS		
				4	6	8
		gm.	%	%	%	%
6	EW (egg white)	151	76	51	49	48
17	EW	201	35	63	74	83
6	EW, autoclaved 6 hours	356	8	..	..	..
17	EW, autoclaved 6 hours	449	30	..	..	..
7	EW, autoclaved 6 hours pH 9	413	..	..	..	..

which time the material was removed from the autoclave, dried and ground. The alkaline autoclaved egg white was prepared by adding sodium hydroxide solution (approximately 10%) to the egg white powder until pH 9 was reached as determined by the hydrogen electrode. This material was then autoclaved for 6 hours at 15 pounds pressure, dried and ground. The pH after autoclaving was found to be 7.5. Two lots of chicks were fed the regular egg-white diet as a control. Two other lots were fed autoclaved egg white in place of the regular egg white. An additional lot was fed alkaline autoclaved egg white in place of the regular egg white.

The results are presented in table 3. These results showed that after coagulation by autoclaving for 6 hours at 15 pounds

pressure at pH 5.9 to 6.0 and at pH 9 none of the pellagra-like syndrome developed. These results are in agreement with the work of Boas-Fixsen ('31) and of Parsons ('31) who finally concluded that cooking destroyed a toxic factor present in raw egg white. A more probable explanation of this, however, as pointed out in the introduction, is that the protective factor was formed or released by cooking.

SUMMARY

Evidence has been presented which shows that dried pork liver contains a factor required to prevent the development of a pellagra-like syndrome in chicks fed an egg-white diet and a factor required for the growth of chicks fed a purified-casein diet. This growth-promoting factor was soluble in an alcohol-water solution, was stable to heating in a dry atmosphere and relatively stable to autoclaving at pH 11. On the other hand, the pellagra-preventing factor was insoluble in an alcohol-water solution, was destroyed by heating in a dry atmosphere and by autoclaving at pH 11 but not at pH 9. This demonstrates that the pellagra-preventing factor is separate from the growth-promoting factor. The growth-promoting factor is vitamin G.

Evidence has also been presented which strongly indicates that the pellagra-like syndrome which develops in chicks fed the egg-white diet is identical with that which develops to a lesser extent in chicks fed the purified casein diet.

When egg white was autoclaved for 6 hours at 15 pounds pressure at pH 5.9 to 6.0 or pH 9, it no longer caused the development of the pellagra-like syndrome. It is believed the more probable explanation of this is that the pellagra-preventing factor is formed or released during autoclaving rather than that a toxic factor is destroyed, in view of the apparent identity of the pellagra-like syndromes and since raw egg white is almost the only source of protein used by the developing chick embryo.

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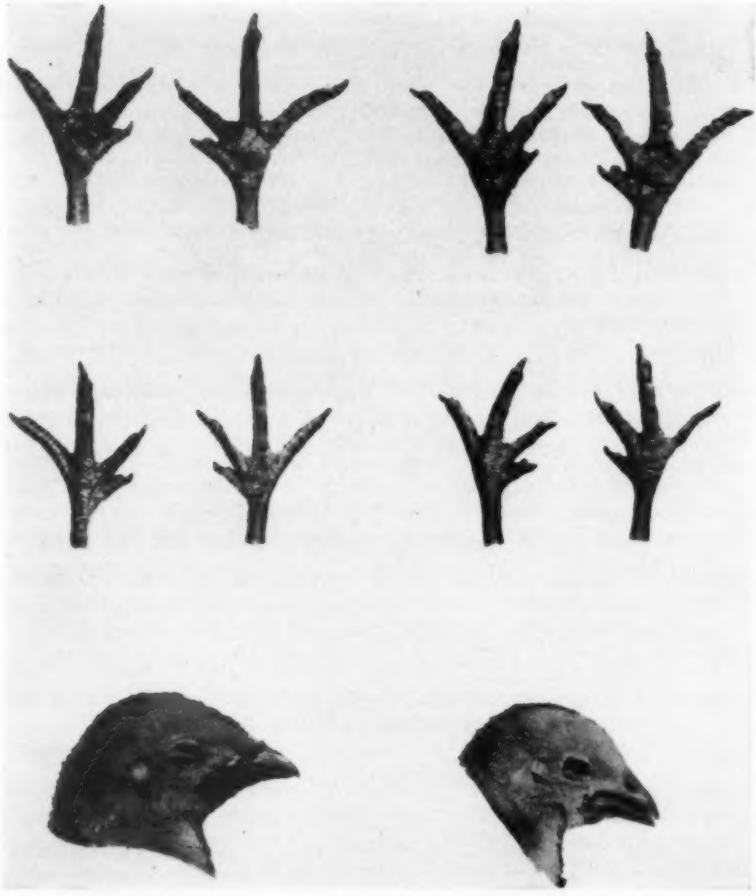

Striking similarity of the pellagra-like syndrome which developed on the egg-white and casein diets. Chick at the left fed the egg-white diet, that at the right the purified-casein diet.

DIFFERENTIATION BETWEEN VITAMIN G AND A SOLUBLE FACTOR PREVENTING A PELLAGRA-LIKE SYNDROME IN CHICKS[1]

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Evidence was presented by Ringrose and Norris ('36) which showed that a factor preventing the development of a pellagra-like syndrome in chicks fed an egg-white diet was not vitamin G(B_2), since this factor did not promote growth in chicks fed a purified-casein diet deficient in this vitamin and was insoluble in an alcohol-water mixture in which vitamin G is soluble. Evidence was also presented in this paper which indicated that the pellagra-like syndrome which developed on the egg-white diet was identical with that which developed to a lesser extent on the purified-casein diet. In a paper by Kline, Keenan, Elvehjem and Hart ('32) it was reported that chicks fed a casein diet heated in a dry atmosphere developed a pellagra-like syndrome similar in appearance to that obtained by Ringrose, Norris and Heuser ('31) in chicks fed either the egg-white or the purified-casein diet. In this work Ringrose, Norris and Heuser found that autoclaved yeast was effective in preventing the syndrome on the casein diet but later Ringrose and Norris ('36) found that yeast, dried or autoclaved, and liver extract were ineffective in preventing it on the egg-white diet. Dried pork liver and the residue of extracted liver on the other hand were completely effective in this respect.

[1] The research reported in this paper was done in partial fulfillment of the requirements for the degree of doctor of philosophy at Cornell University by A. T. Ringrose and is a part of an experiment station project on the vitamin G requirements of poultry under the direction of L. C. Norris.

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Kline et al. ('32) reported that autoclaved yeast and liver extract prevented the syndrome on their heated casein diet. Therefore, in view of the apparent identity of the pellagra-like syndrome which developed on these various diets and the discrepancy in the results on means of preventing it, studies were undertaken on the possibility that two factors were required for its prevention. In conducting these studies evidence was obtained that vitamin G was a complex consisting of two factors. The results of this discovery of which preliminary reports (Ringrose and Norris, '34; Norris and Ringrose, '35) have been made are presented in this paper together with those showing that two factors are required to prevent the development of the pellagra-like syndrome in chicks.

EXPERIMENTAL PROCEDURE

In these studies single comb White Leghorn chicks of the Cornell strain were used. The experimental period was of 8 weeks' duration. Each lot contained twenty day-old chicks at the start. Individual weights were taken weekly together with observations of the pellagra-like syndrome, the procedure being the same as that described by Ringrose and Norris ('36).

The heated casein diet used in this work was similar in composition to the purified-casein diet used previously by Ringrose and Norris ('36). The cornmeal, wheat flour middlings and purified casein were heated by placing in pans at a depth of approximately $\frac{3}{4}$ inch in an oven for 144 hours at 95 to 100°C. After the heating was completed the ration was mixed in the proper proportions, using amounts corresponding to the original material and adding the cod liver oil and minerals on the regular air dry basis. After heating this diet was lacking in a pellagra-preventing factor as well as the growth-promoting factor.

The first experiment with this heated diet was to determine the amount of dried liver necessary to prevent the development of the pellagra-like syndrome and to give normal growth. Dried liver was added to this diet at levels of 0, 1.5, 3.0, 4.5, 6.0 and 7.5%. These additions were made by reducing the

amount of casein in the diet with the increase in the amount of dried liver so that the protein content of the diet was maintained at 25% moisture free basis or approximately 22% air-dry basis. The amount of cornmeal was varied with each liver addition sufficiently to make the whole equal to 100.

The results of this experiment are presented in table 1. The chicks placed on the control heated casein diet grew slowly throughout the entire 8-week experimental period. Between the second and third weeks symptoms of the pellagra-like syndrome began to develop at the corners of the mouth and on the eyelids. These lesions gradually increased in severity and the mortality at the end of the experiment was 100%.

TABLE 1

Quantity of liver required to prevent pellagra developed on the heated purified-casein diet

EXPERIMENT NO.	LOT	GROWTH, WEEKS 8	MORTALITY, WEEKS 8	PELLAGRA, WEEKS		
				4	6	8
		gm.	%	%	%	%
15	HCD (heated casein diet)	..	100	74	100	..
15	HCD — 1.5% dried liver	394	25	16	8	..
15	HCD — 3.0% dried liver	615	15	..	...	..
15	HCD — 4.5% dried liver	637	35	..	...	..
15	HCD — 6.0% dried liver	688	5	..	...	..
15	HCD — 7.5% dried liver	690	35	..	...	..

Insofar as could be determined by a macroscopic examination, the pellagric lesions which developed on the heated casein diet were the same as those obtained previously by Ringrose, Norris and Heuser ('31) on either the egg-white or purified-casein diet, but the general character of the syndrome differed somewhat, possibly because of the presence of a multiple deficiency. When the heated casein diet was fed, the most severe lesions developed at the corners of the mouth. Granulation and sticking together of the eyelids, however, were severe and appeared with great regularity but the lesions on the feet, while they were occasionally as severe as on the egg-white diet, did not appear as regularly.

The feathers of the birds on this diet showed marked hemorrhages at the point where the feathers protrude from the follicles. The main shaft of the feathers became constricted at this point and the remaining portion of the shaft showed a lack of blood supply. These feathers eventually were lost. At about the time that definite loss of feathers was observed the skin became dry, roughened and scaly. By this time the lesions at the corners of the mouth had become large crusty scabs extending often times over the entire area under the lower mandible. The eyelids became severely granulated and the exudate caused the eyelids to stick together so that the affected chicks could not see. Quite commonly, too, lesions developed on the skin around the vent. When the general condition had become this severe death soon followed.

When the heated diet contained 1.5% of dried liver most of the pellagra-like lesions were prevented but mortality was fairly great and growth was not normal. When it contained 3.0% of dried liver, all lesions were prevented and growth almost equal to that on the higher levels of liver was attained. Because of these results it was decided to feed dried liver at the 3.0% level in further work with the heated casein diet.

The purified-casein diet fed to determine the presence of the growth-promoting factor and all supplements used with it were prepared in the same way as those used in work previously reported (Ringrose and Norris, '36). However, the quantity of dried liver fed was 1.5% instead of 2.5%, in order that this slightly sub-optimum level would make it possible to better interpret the results. Additions to both the heated casein diet and the purified-casein diet included dried liver, liver extract, liver residue and liver autoclaved at pH 11.

Two duplicate series of experiments were conducted on the purified-casein diet and the heated casein diet. The first series included experiments 15 and 17 and the second series experiments 18 and 19. The ingredients, such as cereals, purified casein and liver supplements used in the diets of the first series of experiments were from the same supply of ingredients while those used in the second series were from a new supply.

The results of the duplicate series of experiments on both the purified-casein and heated casein diets are summarized in table 2. They show that the chicks fed the purified-casein diet grew very slowly in both series and only moderate mortality

TABLE 2

Differentiation of the growth-promoting factor from the pellagra-preventing factor

EXPERIMENT NO.	LOT	GROWTH, WEEKS 8	MORTALITY, WEEKS 8	PELLAGRA, WEEKS		
				4	6	8
		gm.	%	%	%	%
15	PCD (purified casein diet)	219	30	..	..	..
18	PCD (purified casein diet)	235	30	..	..	..
15	PCD + 1.5% liver	629	..	..	..	..
18	PCD + 1.5% liver	534	..	..	..	..
15	PCD + liver extract [1]	504	15	..	..	..
18	PCD + liver extract [1]	484	..	..	..	..
15	PCD + liver residue [1]	354	5	..	..	..
18	PCD + liver residue [1]	244	..	..	..	..
15	PCD + heated liver [1, 3]	531	..	..	..	..
18	PCD + heated liver [1, 3]	417	5	..	..	..
15	PCD + A liver [1, 4]	239	25	..	..	..
18	PCD + A liver [1, 4]	470	5	..	..	..
17	HCD (heated casein diet)	103	75	52	62	67
19	HCD (heated casein diet)	106	75	47	65	72
17	HCD + 3.0% liver	657	5	..	..	..
19	HCD + 3.0% liver	509	7	..	..	..
17	HCD + liver extract [2]	438	30	..	..	..
19	HCD + liver extract [2]	218	63	18	42	30
17	HCD + liver residue [2]	147	65	33	53	57
19	HCD + liver residue [2]	141	75	47	33	42
17	HCD + heated liver [2, 3]	131	60	44	61	58
19	HCD + heated liver [2, 3]	117	81	49	64	100
21	HCD + heated liver [2, 3]	198	61	36	43	43
17	HCD + A liver [2, 4]	103	70	43	61	59
19	HCD + A liver [2, 4]	249	25	..	..	..

[1] Equivalent to 1.5% liver.

[2] Equivalent to 3.0% liver.

[3] Liver heated for 50 hours at 120°C.

[4] Liver autoclaved at pH 11 for 6 hours at 15 pounds.

[5] Fed at 12% level.

occurred while on the heated casein diet growth was even slower and severe pellagra-like lesions developed accompanied by very high mortality comparable to that of the chicks fed the heated casein diet in the previous experiment.

The results showed that 1.5% dried liver added to the purified-casein diet was sufficient to promote nearly normal growth in both series of experiments. No mortality occurred. The addition of 3.0% dried liver to the heated casein diet gave comparable growth in both series of experiments. None of the pellagra-like syndrome developed and little or no mortality occurred. However, the liver used in the first series gave better growth on both diets than did that of the second one.

The results where liver extract replaced the dried liver in the purified-casein diet showed that good growth was attained by the chicks on this diet in both series of experiments, while the chicks which were fed this diet containing the extracted liver residue grew very slowly attaining an average weight only slightly better than the purified-casein control.

Similarly where liver extract replaced dried liver in the heated casein diet good growth was attained by the chicks on this diet in the first series of experiments. Mortality was not high and none of the syndrome developed. In the second series where liver extract replaced the dried liver in the heated casein diet growth was not quite as good, mortality was higher and some of the pellagra-like lesions developed. In both series the chicks which were fed the heated casein diet containing liver residue grew but little better than those on the heated casein control. Also the syndrome was practically as severe and mortality was as great. This showed that none of the pellagra-preventing factor remained in the extracted liver residue in either series. Therefore, the small amount of pellagra-like syndrome which developed in the liver-extract lot in the second series must have been due to destruction in the preparation of the extract or by some oxidative or other process after the extract was incorporated into the diet.

The results obtained in this series of experiments by feeding liver extract and liver residue show that both the pellagra-preventing factor and the growth-promoting factor are soluble in the alcohol-water mixture. This is in striking contrast to the results obtained in the comparative studies with the egg-white and purified-casein diets (Ringrose and Norris, '36)

which showed that only the growth-promoting factor was soluble in the alcohol-water mixture and that the pellagra-preventing factor remained in the extracted liver residue. It is evident, therefore, that the factor which prevents the pellagra-like syndrome developed on the egg-white diet is different from that which prevents the pellagra-like syndrome developed on the heated casein diet. This difference in solubility between these two factors is in agreement with the work with chicks of Lease and Parsons ('34) published after the completion of this work. Since the pellagric symptoms developed on these diets appeared identical, it seemed probable that two factors were concerned in the prevention of this syndrome.

Whenever liver heated in a dry atmosphere for 50 hours at 120°C. displaced the dried liver in the purified-casein diet good growth was attained but when the heated liver replaced the dried liver in the heated casein diet growth was very poor, severe pellagra-like lesions developed and high mortality occurred. An additional lot fed the heated casein diet with 12% of dried heated liver was included in a later experiment to determine whether or not this dry heat treatment destroys completely the pellagra-preventing factor of liver and that an addition of a larger quantity of the growth-promoting factor contained therein would not prevent the pellagra-like lesions. These results showed that heating liver in a dry atmosphere destroyed the pellagra-preventing factor completely, whereas, the growth-promoting factor was not materially affected by this treatment. The effect of dry heat treatment on the growth-promoting factor is in accord with results previously presented (Ringrose and Norris, '36). It is evident, also, that in spite of the difference in solubility both pellagra-preventing factors are destroyed by heating in a dry atmosphere.

The results where the purified-casein diet containing liver autoclaved at pH 11 was fed showed that the growth-promoting factor may be destroyed by this treatment since the growth in one series, although not in the other, was materially reduced

as compared to that on dried liver. When liver autoclaved at pH 11 replaced dried liver in the heated casein diet growth was poor in both series. In the lot of the first series fed alkaline autoclaved liver the pellagra-like syndrome was severe and the mortality was high but in the comparable lot of the second series no pellagric lesions developed and the mortality was less. These results showed that under certain conditions both the growth-promoting and the pellagra-preventing factors are sensitive to autoclaving at pH 11. The lack of uniformity in results where alkaline autoclaved liver was used indicated, however, that although the initial pH and the pH after autoclaving was the same in both series, either complete uniformity in the alkaline autoclaving procedure was not accomplished or that greater protection was afforded the growth-promoting and the pellagra-preventing factors by one supply of liver than by the other.

The results of this duplicate series of experiments showed that the factor which prevented the pellagra-like syndrome on the heated casein diet is different from the one which promoted growth on the purified-casein diet, since the pellagra-preventing factor was destroyed by heating in a dry atmosphere while the growth-promoting factor was relatively stable to this treatment. Both factors were sensitive to autoclaving at pH 11 and both were soluble in an alcohol-water mixture.

In the final experiment another method of separating the growth-promoting factor from the soluble pellagra-preventing factor was attempted, using both the heated casein diet and the purified-casein diet. It involved the possibility of a differential adsorption of these factors by fuller's earth. This method was based primarily upon the work of Kuhn, György and Wagner-Jauregg ('33) who obtained some evidence that two factors were required for growth and the prevention of skin lesions in rats and that one of these was adsorbed by fuller's earth while the other was not.

The pellagra-preventing and the growth-promoting factors were extracted from liver by the 20% alcohol-water mixture and treated with fuller's earth. Extract equivalent to 500 gm.

of the original dried liver was diluted to 4 liters, acidified with 150 cc. concentrated HCl (37%) and treated with 200 gm. of Surrey fuller's earth. The mixture was stirred constantly for 1 hour after being chilled in an ice bath to a temperature of 10°C. or below. The adsorption was carried out in the cold in order that it might be more complete. After stirring, the fuller's earth was allowed to settle and the supernatant liquid was siphoned off. The fuller's earth was then thrown out by centrifuging and washed three times with distilled water to free it of HCl. The centrifugate and washings were added to the supernatant solution which was treated a second time with 200 gm. of fuller's earth, stirred for 1 hour, and allowed to settle. The supernatant liquid was again siphoned off and the earth thrown out by centrifuging and washed free of HCl. The total 400 gm. of earth was suspended in a solution of 1200 cc. water, 600 cc. methanol and 600 cc. pyridine (practical grade) by stirring and allowed to come to room temperature for elution (summer room temperature 25 to 30°C.).

The elution of the growth-promoting factor from the fuller's earth was completed by repeated extractions with the pyridine-methanol-water solution until the supernatant liquid was colorless. This solution was then concentrated under a vacuum of 650 to 700 mm. until 2 cc. was equivalent to 1 gm. of original dried liver. This preparation, called fraction 1, adsorbable, was then ready for feeding.

The combined supernatant liquid with the washings added were placed in a 12-liter flask and NaOH was added until the solution was slightly alkaline to Congo red but acid to litmus. This solution was then concentrated under a vacuum of 650 to 700 mm. so that 2 cc. was equivalent to 1 gm. of the original dried liver. This preparation, called fraction 2, non-adsorbable, was also ready for feeding.

In the purified-casein diet the supplements were fed at a level equivalent to 2.0% of dried liver and at a level equivalent to 4.0% dried liver in the heated casein diet. Of the five lots of chicks fed the purified-casein diet, one lot served as control and the other lots received additions of liver extract, fraction 1,

adsorbable, fraction 2, non-adsorbable, and fraction 1 and 2 re-combined. Of five lots of chicks fed the heated casein diet, one lot served as control and the others received the same supplements as the lots on the purified-casein diet.

The results of this experiment are presented in table 3. The purified-casein diet as usual gave only slow growth while the heated casein diet also gave very slow growth accompanied by severe pellagra-like lesions and high mortality. When liver extract was combined with these two diets, good growth took

TABLE 3

Differentiation of the growth-promoting factor from the pellagra-preventing factor

EXPERIMENT NO.	LOT	GROWTH, WEEKS 8	MORTALITY, WEEKS 8	PELLAGRA, WEEKS		
				4	6	8
		gm.	%	%	%	%
21	PCD (purified casein diet)	276	6	..	..	..
21	PCD + liver extract [1]	558	..	..	..	..
21	PCD + fraction 1 [3, 3]	539	..	..	..	..
21	PCD + fraction 2 [3, 4]	249	11	..	..	..
21	PCD + fraction 1[3] and 2[3, 4]	554	..	..	..	..
21	HCD (heated casein diet)	98	72	42	62	56
21	HCD + liver extract [2]	406	45	..	..	..
21	HCD + fraction 1 [3, 3]	128	47	41	65	52
21	HCD + fraction 2 [3, 4]	143	7	12	22	25
21	HCD + fraction 1[3] and 2[3, 4]	370	31	..	..	..

[1] Equivalent to 2.0% liver.
[2] Equivalent to 4.0% liver.
[3] Adsorbable fraction.
[4] Non-adsorbable fraction.

place showing again that the liver extract contained both the growth-promoting and the pellagra-preventing factors.

When fraction 1, adsorbable, was combined with the purified-casein diet, good growth equal to that of the original liver extract was obtained but when this fraction was combined with the heated casein diet growth was very poor, mortality was high and the syndrome was as severe as on the heated casein diet only. These results showed that fraction 1, adsorbable, contained the growth-promoting factor but was lacking in the pellagra-preventing factor.

When fraction 2, non-adsorbable, was combined with the purified-casein diet, growth no better than that on the purified-casein diet only was obtained. When fraction 2, non-adsorbable, was combined with the heated casein diet growth was likewise very poor and little mortality occurred. However, a small amount of the pellagra-like syndrome developed on this diet but much less than the amount which occurred when fraction 1, adsorbable, was fed. The symptoms of the syndrome which occurred were the same as far as could be determined as the symptoms in the chicks on the heated casein diet supplemented with fraction 1, adsorbable. This indicated that possibly some destruction of the pellagra-preventing factor took place in the preparation of fraction 2. These results showed that fraction 2, non-adsorbable, contained the pellagra-preventing factor but was lacking in the growth-promoting factor.

When fraction 1 and fraction 2 were re-combined and fed in the purified-casein diet, growth equal to the original liver extract and to fraction 1 alone was obtained. Likewise, when the re-combined fractions were fed with the heated casein diet, good growth took place and none of the pellagra-like lesions occurred.

Thus, additional evidence which demonstrated a definite separation of the growth-promoting factor from the pellagra-preventing factor was obtained, since the growth-promoting factor was adsorbed from liver extract by fuller's earth while the pellagra-preventing factor was not adsorbed.

These results are in agreement with those of Elvehjem and Koehn ('35) which showed that chicks required a factor for the prevention of the pellagra-like syndrome on the heated casein diet which remained in a liver extract after treatment with fuller's earth. They did not find that a separate growth-promoting factor was also required, although they showed that the growth-promoting factor (flavin) adsorbed by fuller's earth from liver extract did not prevent the occurrence of the pellagra-like syndrome. Chick, Copping and Edgar ('35) and György ('35) have reported a factor (flavin) necessary

for the normal nutrition of the rat adsorbable on fuller's earth and another factor non-adsorbable. Both factors were growth-promoting but the non-adsorbable factor was required to prevent rat pellagra while the adsorbable factor was required to prevent a mild dermatitis. Lepkovsky and Jukes ('35) have also reported a growth-promoting factor adsorbed by fuller's earth and a non-adsorbable pellagra-preventing factor required by chicks fed a heated casein diet.

In connection with the work on the heated casein diet it seemed desirable to determine whether the pellagra-preventing factor was contained in the cereal portion (cornmeal and wheat flour middlings) of the diet or in the casein or both.

TABLE 4

Effect of heating in a dry atmosphere on cereals and casein

EXPERIMENT NO.	LOT	GROWTH, WEEKS 8	MORTALITY, WEEKS 8	PELLAGRA, WEEKS		
				4	6	8
		gm.	%	%	%	%
12	Commercial casein + unheated cereals	313	...	..	..	..
12	Commercial casein + heated[1] cereals	...	100	32	49	..
12	Commercial casein heated[1] + unheated cereals	360	35	..	..	..
12	Commercial casein heated[1] + heated[1] cereals	...	100	24	34	..

[1] Heated for 144 hours at 100°C. in a dry atmosphere.

In order to do this four lots of twenty-five day-old chicks per lot were used. The first lot was fed commercial casein combined with unheated cereals, the second commercial casein with heated cereals, the third commercial casein heated with unheated cereals and the fourth commercial casein heated with heated cereals. In this experiment the heated materials were heated in a dry atmosphere at 100°C. for 144 hours, the same as the heated casein diet.

The results of this experiment are presented in table 4. The lot fed the commercial casein with unheated cereals made a fair rate of growth. The lot fed commercial casein with

heated cereals grew very slowly. The mortality was 100% before the end of the eighth week and the pellagra-like syndrome was very severe. When the heated commercial casein with unheated cereals was fed, the growth was as good as the lot which was fed unheated commercial casein with unheated cereals and no pellagric lesions developed but mortality was fairly high. Heated commercial casein with heated cereals gave very slow growth. The syndrome was severe and 100% mortality occurred by the end of the seventh week. It was evident then, that the pellagra-preventing factor was contained in the cereals of the diet and not in the casein.

In view of the fact that it was shown that the factor which prevented the pellagra-like syndrome on the casein diets was contained in the cereals (cornmeal and wheat flour middlings), the ones also used in the egg-white diet, and since the pellagra-like syndrome no longer developed when the egg white was coagulated by autoclaving (Ringrose and Norris, '36), it seemed desirable to determine the effect of heating the coagulated egg white in a dry atmosphere and feeding it in the presence of both heated and unheated cereals.

This experiment contained six lots of twenty chicks each, three of which were fed egg white combined with unheated cereals and three of which were fed egg white combined with heated cereals. Of the three lots fed unheated cereals one lot received raw egg white, the second egg white autoclaved 6 hours at 15 pounds pressure and the third egg white autoclaved 6 hours at 15 pounds pressure and then heated for 50 hours at 120°C. Likewise of the three lots fed heated cereals one lot received egg white, the second egg white autoclaved and the third egg white autoclaved and heated.

The results are presented in table 5. The lot fed egg white with unheated cereals, the regular egg white control, gave the usual results of poor growth, high mortality and severe pellagra-like lesions. With autoclaved egg white and unheated cereals the growth was fairly good, with no lesions and no mortality. With egg white autoclaved and heated plus unheated cereals growth was not as good as that on autoclaved

egg white plus unheated cereals but no pellagra-like lesions developed and mortality was slight.

On the diet containing egg white and heated cereals again growth was very poor, the pellagra-like syndrome was as severe as that on the regular egg-white diet and mortality was twice as great. Autoclaved egg white with heated cereals gave poor growth, a small amount of pellagra-like lesions and mortality twice that on the regular egg-white diet. Heated autoclaved egg white with heated cereals gave very slow growth and mortality almost twice that on the regular egg-white diet. The pellagra-like syndrome developed earlier

TABLE 5

Effect of heating in a dry atmosphere on autoclaved egg white

EXPERIMENT NO.	LOT	GROWTH, WEEKS 8	MORTALITY, WEEKS 8	PELLAGRA, WEEKS		
				4	6	8
		gm.	%	%	%	%
17	EW (egg white)	201	35	63	74	83
17	EW, A 6 hours	449	..	..	..	..
17	EW, A then heated[2]	330	10	..	..	..
17	EW[1]	153	70	54	86	87
17	EW, A 6 hours[1]	133	80	..	22	17
17	EW, A then heated[1, 2]	102	60	15	29	32

[1] Cereals heated 144 hours at 100°C. dry.

[2] Autoclaved 6 hours then heated 50 hours at 120°C. dry.

A = autoclaved.

and was slightly more severe than on the autoclaved egg white with heated cereals. The dry heat treatment when applied to the autoclaved egg white was evidently not as effective in destroying the pellagra-preventing factor as when applied to cereals and dried liver.

This experiment indicated that the factor which prevented the pellagra-like syndrome resulting from heating the cereals is contained in fairly large amounts in autoclaved egg white, since when the autoclaved egg white was fed the development of pellagric lesions was almost entirely prevented. Whether the factor concerned with preventing the pellagra developed by

heating cereals was contained in raw egg white could not be determined from these results, since in the lot fed raw egg white with heated cereals both pellagra-preventing factors may have been lacking, as it was possible that both may have been formed or released in autoclaving egg white. No differences in type of pellagric lesions could be detected in this lot which is added evidence that the two syndromes are the same. Whether one or both pellagra-preventing factors were partially destroyed by dry heating autoclaved egg white could not be determined, since dry heat treatment has been shown to destroy both factors.

In view of the fact that the pellagra-like syndrome which developed in chicks fed the egg-white diet (Ringrose and Norris, '36) appeared identical with the pellagra-like syndrome developed in chicks fed the heated casein diets it seemed evident that two factors were required to prevent the development of this syndrome, since one was insoluble in an alcohol-water mixture while the other was soluble. It has been shown that autoclaved egg white contained the factor which prevented the syndrome on the heated casein diet and Boas-Fixsen ('31) and Gorter ('35) showed that purified casein contained the factor which prevented the syndrome on the egg-white diet.

It is interesting to note in this connection that Salmon ('31) observed in work with rats fed a synthetic diet containing casein heated in a dry atmosphere for 45 hours at 130° to 140°C. a pellagra-like syndrome very similar to the pellagra-like syndrome observed by Boas ('27), Parsons ('31) and Salmon and Goodman ('34) in rats fed egg-white diets. From the evidence presented herein it appeared possible that Salmon destroyed the factor in casein which prevents the pellagra-like syndrome developed on raw egg-white diets, since, it has been shown that this factor is destroyed by dry heating and that casein lacks the factor which prevents the pellagra-like syndrome developed on the heated casein diet.

SUMMARY

Evidence has been presented which shows that dried pork liver contains a factor required to prevent the development of a pellagra-like syndrome in chicks fed a heated casein diet and a factor required for the growth of chicks fed a purified-casein diet. Both of these factors are soluble in an alcohol-water mixture and sensitive to autoclaving at pH 11.

The pellagra-preventing factor was destroyed by heating in a dry atmosphere for 144 hours at 100°C. or 50 hours at 120°C. while the growth-promoting factor was relatively stable to this treatment. The pellagra-preventing factor was not adsorbed from liver extract by fuller's earth while the growth-promoting factor was adsorbed. Vitamin G is, therefore, a complex consisting of two components, one of which is pellagra-preventing while the other of which is essentially growth-promoting.

The pellagra-preventing factor was found to be present in the cereals only of a cereal, casein diet and not in the casein. Coagulated egg white was shown to contain the pellagra-preventing factor but whether raw egg white also contained this factor could not be determined from the results.

Further evidence was obtained which indicated the identity of the pellagra-like syndrome which develops in chicks fed the heated casein diet with that which develops in chicks fed a raw egg-white diet. Two factors were required to prevent the development of this syndrome, since one of these was soluble in an alcohol-water mixture while the other was insoluble. One was also more sensitive to autoclaving at pH 11 than the other but both were destroyed by prolonged heating in a dry atmosphere.

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THE DISTRIBUTION AND PROPERTIES OF THE ANTI-GIZZARD-EROSION FACTOR REQUIRED BY CHICKS [1]

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The occurrence in chicks of a 'scurvy-like syndrome' of dietary origin was described in 1933 by Holst and Halbrook and in 1934 by Dam and Dam and Schönheyder. This condition was characterized by hemorrhage, anemia, and crater-like lesions in the gizzard lining, and was prevented according to Dam and Schönheyder by feeding hog liver, hemp seed, tomatoes or kale. The factor could be extracted from these materials with ether. In subsequent papers by Dam ('35) the ability of various dietary constituents to induce normal blood clotting time has been taken as a criterion of their potency in the antihemorrhagic factor and little has been said about the gizzard lesions. In 1935 Almquist and Stokstad published the results of their investigations on the antihemorrhagic factor and expressed the opinion that the hemorrhagic condition and the gizzard lesions were not manifestations of the same deficiency since the former often occurred in the absence of the latter and vice versa. We began our studies of the gizzard erosions because it was found that this condition was a

[1] Published with the permission of the director of the Wisconsin Agricultural Experiment Station.

complicating factor in our experiments on vitamin B_4 deficiency in chicks (Kline et al., '36). The results of these studies were presented at the meetings of the American Society of Biological Chemists in March, 1936 (Bird et al., '36). It was stated that the anti-gizzard-erosion factor occurred abundantly in pork lung, liver and kidney, from which sources it was not extractable by ether, ethyl alcohol or water. The factor was brought into solution by treating fresh pork lung with 0.3% NaOH and upon precipitation of the proteins by acidification to pH 4.5 was found in the protein precipitate. Oats and wheat were listed as intermediate sources of the factor and alfalfa, wheat seedlings, yellow corn, corn oil, wheat germ oil, soy bean oil, and peanuts as poor sources. The factor as it occurred in grains was found to be quite labile both to dry heat at 120°C. and to autoclaving, but was somewhat more stable in lung tissue. The insolubility of the gizzard factor in ether was presented as proof of its non-identity with the antihemorrhagic factor.

Subsequently Almquist and Stokstad ('36) reported that the gizzard lesions were prevented by feeding high levels of a hexane extract of dried kale or dried alfalfa, and that upon saponification the activity was found in the saponifiable fraction. Since the antihemorrhagic factor remains in the unsaponifiable fraction, the conclusion is drawn that the two factors are distinct. Since these results are at variance with ours, in that we found alfalfa to be a poor source of the gizzard factor and the gizzard factor to be insoluble in fat solvents, it seemed advisable to publish the details of our experiments.

EXPERIMENTAL

Day-old chicks were obtained from the department of poultry husbandry for these experiments. Each group was placed in a 14 inch by 16 inch by 14 inch cage individually heated and equipped with a raised wire screen bottom. The ration was supplied in round, covered metal feeders in which the feed was available to the chicks through holes in the cover. For

the most part groups of four chicks were started on the various rations and after 3 weeks each group was reduced to three chicks, and the gizzards of the chicks killed were examined. This practice was followed in order to allow for deaths during the first few days of the experiment and to prevent overcrowding during the last part of the experiment, since the cages used were not large enough for four chicks after the third or fourth weeks. The remaining chicks were killed at 5 weeks of age and the gizzards examined. The gizzards were graded according to the severity of the lesions as none or slight, marked and severe; and the gizzard factor potency of any dietary supplement was judged by its ability to produce gizzards with slight or no lesions as compared with gizzards having marked or severe lesions from the chicks on the basal ration. There are obvious defects in such a method, but it appeared to be the best available. It was not possible to use complete prevention of gizzard erosion as a criterion since we have only rarely found chicks under 6 weeks of age with gizzards entirely free of lesions, even on the best rations. The grading of the gizzards was done by the same person throughout these experiments, thus ruling out the factor of personal variation. The length of the experiments was set at 5 weeks because experiments in which chicks were killed at weekly intervals showed that the lesions continued to increase in severity up to 5 or 6 weeks of age. The greatest increase in severity was during the third week, corresponding to a marked falling off in food consumption.

As stated above, our earlier experiments on the gizzard factor were directed toward preventing gizzard erosion in chicks used in vitamin B_4 experiments. During the process of modification of the vitamin B_4 basal ration, a considerable amount of data on the gizzard factor was accumulated, and some of this material is presented here. These experiments were conducted in essentially the same manner as described above except that the chicks were killed at 6 weeks of age rather than at 5 weeks.

The basal rations used in these experiments are given in table 1. The grain rations 351 and 351E were not sufficiently

TABLE 1

Composition of rations

INGREDIENTS	RATION 351	RATION 351E	RATION 441	RATION 449	RATION 450H	RATION 454	RATION 456
Yellow corn	58						
Yellow corn, autoclaved 15 pounds 5 hours		55					
Standard wheat middlings	25						
Standard wheat middlings, autoclaved 15 pounds 5 hours		25					
Dextrin			53.5	70			
Dextrin, heated 120°C. 24 hours					69	61	60
Crude casein	12		24				
Crude casein, autoclaved 15 pounds 5 hours		12					
Crude casein, heated 120°C. 24 hours					18	18	18
Reprecipitated casein				18			
Baker's yeast	1		8				3
Baker's yeast, ether extracted		4					
Brewer's yeast				1	2	2	2
Liver residue, autoclaved 15 pounds 10 hours			10	2			
Liver residue, autoclaved 15 pounds 10 hours heated 120°C. 24 hours					2		
Liver extract				2	2	2	
NaCl	1	1					
$CaCO_3$	1	1					
$Ca_3(PO_4)_2$	1	1					
Salt mixture 1				5	5	5	5
Salt mixture 40			2.5				
Peanuts						10	10
Cod liver oil	1	1	2	2	2	2	2

low in the gizzard factor to be satisfactory basal rations, but 351 was used with some success in experiments on the heat stability of the factor, the heat treatment being applied to the corn-middlings-casein part of the ration. Rations 441, 449 and 450H were used as basal rations in vitamin B_4 experiments. With the exception of 441 which contains 24% of crude casein they are relatively low in the gizzard factor. Rations 454 and 456 have proved most satisfactory for basal rations in this work. It was found necessary to include 10% of peanuts, as indicated in the table, in order to supply sufficient vitamin B_4. The brewer's yeast, obtained from Premier-Pabst Co. was added primarily as a source of vitamin $B(B_1)$. The liver extract in ration 454 was added primarily as a source of vitamin $G(B_2)$.[2] It had the disadvantages of being very hygroscopic and also rather expensive. In ration 456 the vitamin G requirement was met by including 3% of baker's yeast (Anheuser-Busch, strain C), making the total yeast content 5%.

Representative data on the distribution, stability and solubility of the gizzard factor are presented in tables 2, 3 and 4 respectively. The number of basal rations used made it difficult to summarize briefly the results, and much material has been omitted from these tables for the sake of brevity. Practically all the results presented have been confirmed by experiments with other basal rations.

Table 2 clearly shows the effectiveness of pork lung, liver and kidney as sources of the gizzard factor. The materials designated as 'vacuum dried' were obtained from Dr. David Klein of the Wilson Laboratories, and the other meat products were obtained fresh from a local packing plant and dried at 50°C. Among the grains the superiority of oats is evident, although wheat bran and wheat middlings were also good sources of the anti-gizzard erosion factor and somewhat superior to wheat itself. Dried leafy plant material, represented in the table by alfalfa and wheat seedlings, was not

[2] We are indebted to Dr. Harold Levine, Premier Pabst Corporation, Milwaukee, Wisconsin, for supplying the brewer's yeast and to Dr. David Klein, Wilson Laboratories, for supplying the liver extract.

TABLE 2

Distribution of the gizzard factor

BASAL RATION	SUPPLEMENT	AVERAGE WEIGHT 3 WEEKS	AVERAGE WEIGHT 5 WEEKS	AGE KILLED	CHICKS WITH GIZZARD LESIONS		
					Slight or none	Marked	Severe
		gm.	*gm.*	*weeks*			
441	None	86	148	6	1	5	
441	10% dried wheat seedlings	115	218	6		2	
449	32% wheat	62	102	6			2
449	32% wheat + 5% vacuum dried lung	132	202	6	2		
449	32% yellow corn	92	166	6			2
449	32% yellow corn + 5% vacuum dried lung	115	202	6	2		
450H	None	68	...	4		1	2
450H	12% alfalfa	68	...	4	1	1	1
450H	5% H_2O extracted lung	77	...	4	2	1	
450H	5% H_2O extracted lung + 12% alfalfa	100	...	4	1	2	
454	None	72	130	5	1	4	4
454	15% pork lung	126	265	5	6		
454	15% vacuum dried stomach	97	212	5	2	1	
454	32% whole oats	114	237	5	6		
454	32% hulled oats	97	222	5	2	1	
454	32% rolled oats	125	252	5	2	1	
454	32% wheat	87	200	5	2	1	
454	32% Wisconsin yellow corn	90	188	5		2	1
454	32% Texas yellow corn	88	188	5	1		2
456	None	71	137	5		7	3
456	32% whole oats	129	247	5	9	1	
456	5% gastric mucin granules	90	180	5	2		
456	32% wheat bran	110	222	5	3		
456	32% standard wheat middlings	110	190	5	2	1	
351E	None	131	213	5	2	2	3
351E	15%pork liver	156	304	5	7		1
351E	15%pork kidney	143	307	5	6		
351E	15% pork heart	159	266	5	4	3	1

effective. Other materials, not mentioned in the table, which gave negative results were dried blue grass, peanuts, soy bean oil, and wheat germ oil.

That the factor is destroyed to a considerable extent either by dry heat or by autoclaving is indicated in table 3. The more thorough destruction in grains, than in lung may be due to a difference in the amounts originally present. The factor as it occurs in lung is more labile at a pH of 7.5 than at natural pH.

Table 4 on the solubility of the gizzard factor indicates the complete failure of attempts to extract it with ether, alcohol, or water. The water extract of lung was prepared by adding cold water to the lung tissue, heating to the boiling point and filtering hot. The first success in bringing the factor into solution was achieved by treating fresh pork lung with 0.3% NaOH. The gizzard factor potency appeared in the precipitate resulting when the NaOH solution was acidified to pH 4.5.

Fresh pork lung was also fractionated according to the method of Siegfried ('02) for the preparation of reticulin, it having been noted that the three best sources of the gizzard factor, lung, liver and kidney are also the organs containing the largest amount of reticular connective tissue. The lung tissue was digested with trypsin, the residue extracted with ether, the ether residue boiled 20 minutes with 0.05% HCl, filtered, and the residue washed once with warm water. The filtrate was designated the gelatin fraction and the residue the reticulin fraction. The activity, as shown in the table, appeared in the latter fraction.

An attempt to use the NaOH treatment and subsequent acid precipitation with oats instead of with lung was unsuccessful, the activity being distributed among the three fractions.

TABLE 3

Stability of the gizzard factor

BASAL RATION	MODIFICATION	AVERAGE WEIGHT 3 WEEKS	AVERAGE WEIGHT 5 WEEKS	AGE KILLED	CHICKS WITH GIZZARD LESIONS		
					Slight or none	Marked	Severe
		gm.	*gm.*	*weeks*			
351	None	129	252	5–6	13	1	
351	Heated 60°C. 24 hours	141	251	6	5		
351	Heated 80°C. 24 hours	114	219	5–6	4	9	1
351	Heated 100°C. 24 hours	97	206	5	3	2	4
351	Heated 120°C. 24 hours	93	148	6	1	2	1
441	None	90	158	6		3	
441	24% yellow corn	103	207	6	3		
441	32% yellow corn	93	195	6	2		1
441	24% yellow corn, heated 120°C. 24 hours	90	155	6		3	
441	32% yellow corn, heated 120°C. 24 hours	90	198	6		3	
441	10% vacuum dried lung	109	164	6	3		
441	15% vacuum dried lung, heated 90°C. 24 hours	165	280	6	2		
441	15% vacuum dried lung, heated 120°C. 24 hours	142	250	6	1	2	
454	None	60	123	5		1	2
454	15% vacuum dried lung, heated 120°C. 24 hours	100	217	5		1	1
454	15% vacuum dried lung, autoclaved 15 pounds 5 hours	72	122	5	1	2	

TABLE 4

Solubility of the gizzard factor

BASAL RATION	SUPPLEMENT	AVERAGE WEIGHT 3 WEEKS	AVERAGE WEIGHT 5 WEEKS	AGE KILLED	CHICKS WITH GIZZARD LESIONS		
					Slight or none	Marked	Severe
		gm.	*gm.*	*weeks*			
351E	None	131	213	5	2	2	3
351E	Ether extract of 15% pork liver	114	211	5	1	4	2
351E	Ether extract of 15% pork kidney	104	200	5	2	1	4
351E	Ether extract of 15% pork heart	113	216	5	5	2	2
351E	Ether extract of 10% dried alfalfa	104	181	5	1	3	4
441	Ether extract of 15% vacuum dried lung	100	183	6		3	
441	Ether residue of 15% vacuum dried lung	147	270	6	2		
441	95% alcohol extract of 15% vacuum dried lung	108	201	6		3	
441	95% alcohol residue of 15% vacuum dried lung	137	277	6	3		
450H	None	68	...	4		1	2
450H	5% H_2O extracted residue of vacuum dried lung	77	...	4	2	1	
454	None	74	139	5	1	4	4
454	15% porklung	126	265	5	6		
454	NaOH residue of 15% pork lung	90	177	5		2	1
454	Acid ppt. of NaOH extract of 15% pork lung	79	142	5	6		
454	Acid soluble fraction of NaOH extract of 15% pork lung	60	95	5		1	1
454	Trypsin digest of 15% pork lung	107	190	5		3	
454	Ether extract of trypsin residue of 15% pork lung	73	140	5		2	1
454	Gelatin fraction of 15% pork lung	63	118	5	1	1	1
454	Reticulin fraction of 15% pork lung	85	162	5	3		
456	None	61	112	5		2	1
456	32% whole oats	115	257	5	2		
456	NaOH residue of 32% whole oats	77	143	5	2	1	
456	Acid ppt. of NaOH extract of 32% whole oats	89	162	5	2	1	
456	Acid soluble fraction of NaOH extract of 32% whole oats	84	192	5	3		

DISCUSSION

The divergence in the results obtained with alfalfa by Almquist and Stokstad and by us is difficult to explain, even on a quantitative basis, although there was a considerable difference in the amounts fed. Almquist and Stokstad found that their chicks were protected by the hexane extract equivalent to 25% alfalfa. Upon adding 12% of alfalfa to ration 450H we found only very doubtful improvement in the gizzard lining, and the addition of the same level of alfalfa to ration 450H plus 5% water extracted lung increased rather than decreased the severity of the gizzard lesions. Likewise the addition to ration 351E of the ether extract equivalent to 10% alfalfa seemed to increase the severity of the lesions. The work with leafy plant tissue was, in general, very unpromising since grass and wheat seedlings also tended to increase the severity of the gizzard lesions.

Pork lung has proved thus far to be the most satisfactory source of the factor, and here as in other sources the factor was entirely unextractable by ether. The finding of the gizzard factor potency in the acid precipitable fraction of the NaOH extract of lung, indicates a relation to the proteins, but whether the factor is chemically combined in the protein molecule or merely adsorbed, it is of course impossible to say. The results of the preparation of reticulin from lung tissue show the association to be with the more resistant protein, not hydrolysed by trypsin. The possibility of relationship between the protein of the gizzard lining and the reticulin of lung, liver and kidney leads to interesting speculations; however, the results obtained upon the fractionation of oats show that the factor is not necesssarily a part of a protein molecule.

The superiority of oats over the other whole grains studied as a source of the gizzard factor is likely to be of considerable practical importance. The possibility of improving, under certain conditions, the growth of young chicks by the addition of oats to the ration has been recognized for some time. Wilcke ('36) has recently reported data of this nature. However, so far as is known, no satisfactory explanation of these results

has until now been supplied. Gizzard erosion has been observed quite extensively under practical conditions, which is not surprising in view of the general use of corn, a poor source of the gizzard factor, in poultry rations.

At an early stage in this work, the heat lability of the factor as it occurs in grains suggested the possibility of its identity with vitamin B_2. The later discovery of the greater stability of the factor as it occurs in lung seemed to argue against this possibility, but this greater stability may have been due merely to a greater concentration originally present. Attempts to concentrate the factor from lung by a method similar to that used by O'Brien ('34) for the concentration of B_2 from wheat germ were unsuccessful, but this may have been due to obvious differences in the source material. A pigeon experiment gave inconclusive results although pigeons fed on polished rice supposedly deficient in vitamin B_2 did show slight gizzard lesions. Disagreements in the literature as to the properties of vitamin B_2 make it difficult to establish definitely the identity or non-identity of the two factors. It seems possible that the condition of poor growth in chicks described by Eddy, Gurin and Keresztesy ('30) and supposedly a vitamin B_2 deficiency may have been a gizzard factor deficiency.

CONCLUSIONS

It may be concluded that the anti-gizzard-erosion factor is distinct from the antihemorrhagic factor required by chicks; that it occurs abundantly in pork lung, liver and kidney, and in a descending order of abundance in oats, wheat and corn; that wheat bran and wheat middlings compare favorably with oats as sources of the factor; that it exhibits some variation in heat stability depending on the source; and that it is insoluble in ether and in ethyl alcohol but follows the alkali soluble acid precipitable proteins in the fractionation of lung tissue.

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THE USE OF A 10-DAY PERIOD FOR THE ASSAY OF VITAMIN B BY RAT GROWTH TECHNIC[1]

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SIX FIGURES

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The need for a short time method of assaying vitamin B[2] developed during a study of the vitamin B retentions of children. Because the quantities of urine and feces available from the children during a metabolism period were definitely limited, it was impossible to employ the 8-week, or even 4-week, method of assay recommended by Chase and Sherman ('31). In order to determine whether growth for 10 days is a valid criterion of the amount of vitamin B in the material being tested, a study has been made of the factors which might cause variability in results. While several of these factors have previously been reported by investigators of vitamin B requirements, they have not all been studied and compared under the same technic, nor evaluated in relation to the growth of the animal.

FACTORS AFFECTING THE LENGTH OF THE DEPLETION PERIOD

Ration ingredients. Rats, whose weights were within the range of 40 to 45 gm., were weaned at 21 days of age and placed in individual cages with raised screen bottoms. Groups consisting of three males and three females (no two rats

[1] The preliminary work for this study was done at the Iowa Child Welfare Research Station, Iowa City, Iowa.

[2] The term vitamin B has been used for the fraction of the vitamin B-complex sometimes designated vitamin B_1.

from the same litter) were given either the following ration A or a variation of it.

Ration A

	gm.		gm.
Casein[3]	18.0	Cod liver oil	2.0
Dextrin[4]	60.5	Butterfat (filtered)	5.0
Agar	2.0	Crisco	8.0
Salts[5]	4.5		
Autoclaved yeast[6]	0.5 gm. per day		

Ration A was chosen because of its similarity to the ration recommended by Chase and Sherman. The variations were partly chosen because of the report of Bender, Flanigan and Supplee ('34) in regard to the use of autoclaved whey, and the report of Guerrant, Dutcher and Tomey ('35) in regard to the source of carbohydrate. The changes in the ration were a comparison of autoclaved whey[7] with autoclaved yeast, an increase of the supplement used to supply vitamin G, substitution of sucrose for dextrin, and omission of agar and fat. The rats were weighed weekly and observed daily for symptoms.

The outstanding result was the variation in the length of time before polyneuritis developed and the animals died. Figure 1 records this variation by giving the number of days before death for the individual rats.

[3] Vitamin free casein was obtained from the Casein Mfg. Co., New York.

[4] Tapioca was autoclaved for 4 hours at 16 pounds pressure and ground.

[5] Salts consisted of McCollum no. 185 plus 17.7% calcium carbonate and 2.86% ferric citrate.

[6] Yeast was prepared by mixing 190 gm. Mead Johnson Yeast with 50 cc. N NaOH and 200 cc. water. After standing overnight in a refrigerator the yeast mixture was autoclaved for 5 hours at 16 pounds pressure, dried and ground. This process was then repeated with the single change of only 2 hours autoclaving.

[7] Dried whey (Peebles Lacto Milk) was furnished through the courtesy of the Western Condensing Co., San Francisco. The whey was mixed with an equal weight of distilled water and autoclaved, in shallow pans, for 2 hours, at 16 to 18 pounds pressure.

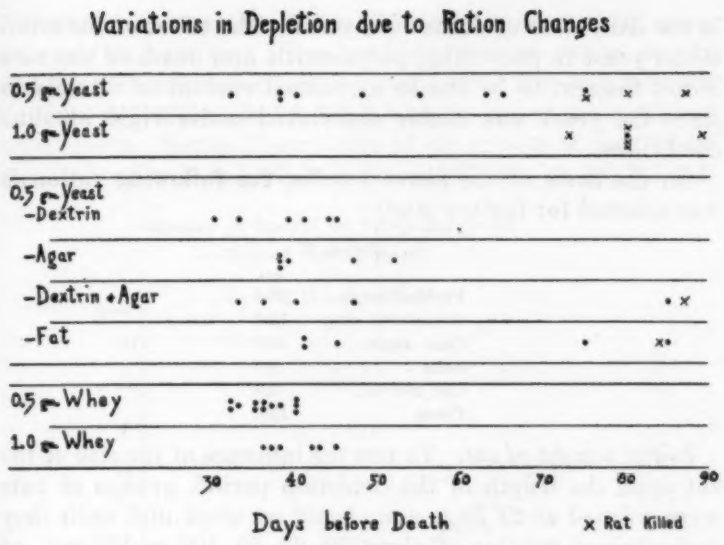

Figure 1

The results may be summarized as follows:

RATION		NUMBER OF RATS		NUMBER OF DAYS BEFORE DEATH	
Supplement	Change	Living	Dead	Range	Average
0.5 yeast	.....	3	9	32–75	51
1.0 yeast	.....	5	1	80–	80
0.5 yeast	No dextrin	0	6	30–45	39
0.5 yeast	No agar	0	6	38–52	42
0.5 yeast	No dextrin or agar	1	5	33–85	48
0.5 yeast	No fat	1	5	41–85	57
0.5 whey	.....	0	12	32–40	36
1.0 whey	.....	0	6	36–45	40

It will be noted that a change in the daily supplement from autoclaved yeast to autoclaved whey produced more uniformity in results than did the substitution of sucrose for dextrin in the ration or the omission of agar. Furthermore, an increase of autoclaved whey from 0.5 gm. daily to 1.0 gm. did not greatly prolong the life of the animal as did a similar increase

in the daily dose of autoclaved yeast. The effect of the additional yeast in preventing polyneuritis and death of the rats is not thought to be due to a residual content of vitamin B since the yeast was double autoclaved under rigid alkaline conditions.

On the basis of the above results, the following ration B was selected for further study:

Ration B

	gm.
Purified casein	20.0
Autoclaved whey	15.0
Cane sugar	38.0
Salts	5.0
Cod liver oil	3.0
Crisco	19.0

Initial weight of rat. To test the influence of the size of the rat upon the length of the depletion period, groups of rats were weaned at 21 days and placed on stock diet until they had attained weights of about 50, 60, 80, 100 or 120 gm., at which time they were transferred to the vitamin B free ration. The average time before death for each group was as follows:

Average initial weight	*Number of rats*	*Average days before death*
43	11	32.0
50	7	37.3
61	18	34.3
81	5	39.2
102	6	35.0
121	6	39.3

While there was not a great deal of difference in results for these different weight groups, a slightly more uniform response was observed for the group averaging about 60 gm. than for smaller or larger rats.

FACTORS AFFECTING THE GROWTH RESPONSE OF THE RATS

Ration ingredients. Following a depletion period of about 3 weeks, doses of stabilized wheat germ,[8] have been fed for

[8] A wheat germ product, stabilized through removal of fat and some moisture, containing approximately 15 Sherman units of vitamin B per gram, has been supplied by the VioBin Corporation, Chicago, Illinois.

8 weeks to two series of rats. Series I consisted of seven groups (six rats to a group) which received ration A. In addition, the animals of these groups were fed daily (except Sunday) 0, 0.0375, 0.05, 0.1, 0.2, 0.5, or 1.0 gm. of the stabilized wheat germ. Series II consisted of six groups of rats which received ration B and wheat germ as follows: 0, 0.1, 0.2, 0.3,

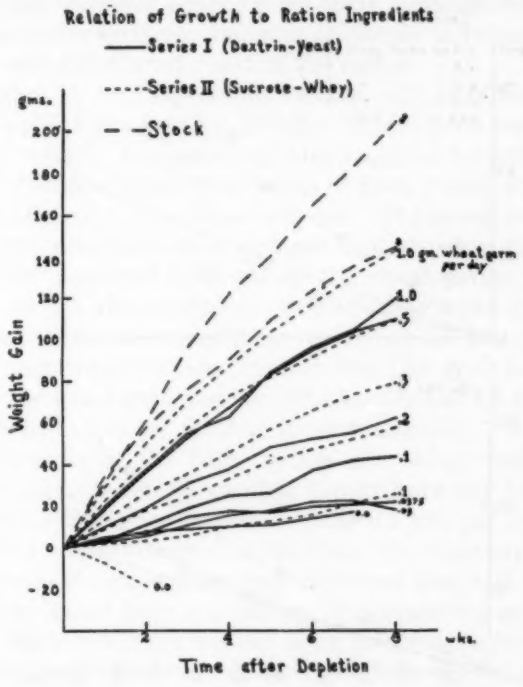

Figure 2

0.5, or 1.0 gm. per day. The growth curves for the 8 weeks after depletion for both series are presented in figure 2. The rats of series I, which had received dextrin and autoclaved yeast, grew better at the lower levels of wheat germ and more poorly at the higher levels than did the rats of series II, which had been fed sucrose and autoclaved whey. Apparently

the yeast-dextrin combination enabled the rats to grow to a certain extent regardless of insufficient quantities of vitamin B in their daily supplements, but was not adequate to support growth when larger amounts of vitamin B were administered.

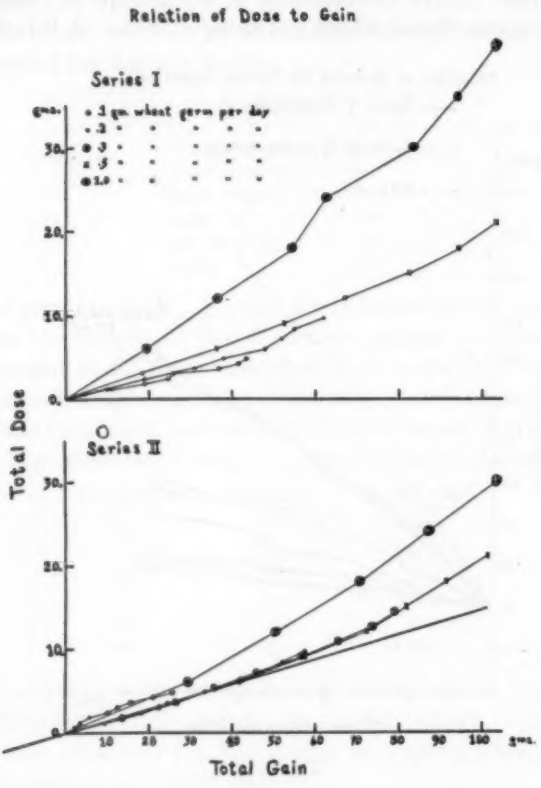

Figure 3

Size of dose. In an attempt to analyze further the growth response of these two series of rats, the total accumulative amount of dose per rat, at the end of each week, for each group, has been plotted in figure 3 against the average accumulative gain for the corresponding period. As has been

previously commented, poor growth resulted in series I in the group receiving 1.0 gm. of wheat germ per day. A study of figure 3 shows that this poor growth was the result of a progressive increase in the amount of dose required for a gram of gain as the ingestion of wheat germ was increased from 0.1 to 0.2, 0.5 and 1.0 gm. per day. The 0.05 and 0.0375 gm. groups were not plotted since their response was similar to the negative controls and was obviously influenced by the dextrin and autoclaved yeast of the ration.

In series II very consistent results were obtained for the 0.2, 0.3 and 0.5 gm. per day groups. While there was a slight upward trend in the amount of dose required for gain toward the end of each of these three levels of dose, a very close direct relation of gain to dose was observed. The lower portions of each curve coincided in a straight line showing that 1 gm. of gain had required 0.145 gm. of the wheat germ. Both the 1.0 and the 0.1 gm. groups required slightly more dose for a unit gain. Since the growth curves of the 1.0 gm. group are nearly comparable to those for stock rats (fig. 2), it is probable that this lessened gain per unit of dose for the 1.0 gm. group was due to a lack of further growth capacity. Additional tests, however, of the basal ration are being conducted to ascertain more definitely whether it may have any limitations for growth. The poor response of the 0.1 gm. per day group may be due to the low level of the dose. In other experiments with vitamin B assays it has been observed that rats receiving border line doses have a tendency to become too greatly depleted. Such rats must restock their body stores to a certain extent before growth is resumed. While the rats of this 0.1 gm. per day group were not depleted to the point of polyneuritic symptoms such as incoordination of movements or paralysis, in all probability they were not receiving at first sufficient vitamin B for regular growth. Gradually, however, their condition improved until at 8 weeks their response approached that for the next two groups. The fact that this inferior response for the 0.1 gm. per day group was not particularly noticeable when the growth curves were plotted

in figure 2, but became apparent only when the amount of gain was analyzed in relation to the dose, would seem an important consideration in evaluating the results of a vitamin B assay.

The relation of gain to dose has therefore been subjected to further analysis. In figure 4 the logarithms of the total

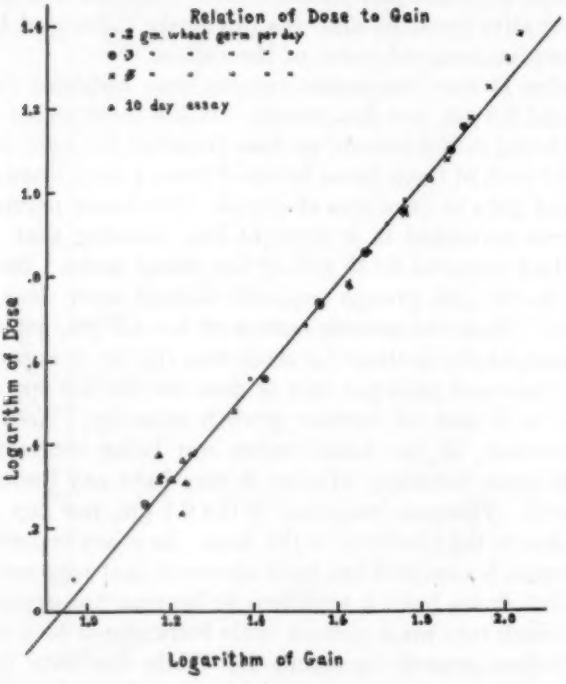

Figure 4

amounts of dose at the end of each week have been plotted against the logarithms of the total gains. This method of analysis brought out a greater degree of direct dependence of gain upon dose than was apparent in figure 3. Nearly all of the slight upward curves observed in figure 3 were eliminated and a longer straight line resulted when the loga-

rithms of dose and gain were used for 0.2, 0.3 and 0.5 gm. groups. Equations developed from the straight line of figure 4 gave the following results:

$$\text{Dose} = 0.0773\ (\text{gain})^{1.786}$$

$$\text{Gain} = \sqrt[1.786]{\text{dose} + 12.95}$$

Weight of rat. Since the total gain at any time appears to be directly related to the accumulative effect of the total dose at that time, the amounts of dose required for 1 gm. of gain have been calculated at the end of each week for the rats of series II. When these dose per gram of gain figures were

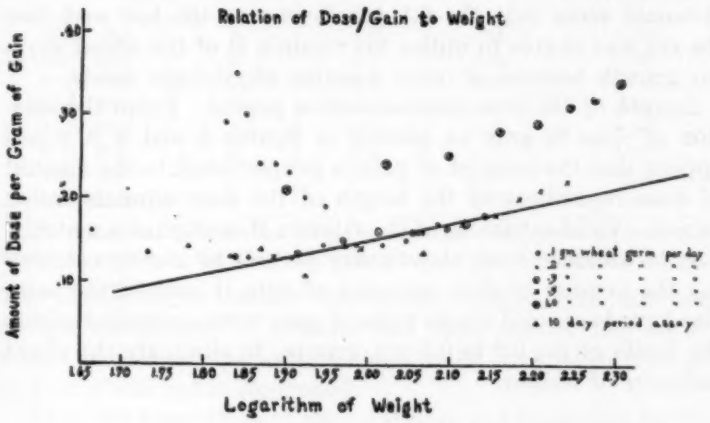

Figure 5

plotted against the average weights of the rats for each week, a series of curved lines resulted. Cowgill ('34) has stated that the vitamin B requirement is related to the 5/3 power of the weight. The dose per gram of gain figures have therefore been plotted in figure 5 against the logarithms of the average weights for each week. This treatment straightened somewhat the curves obtained by use of unaltered weight figures, but did not indicate as direct a relation between dose/gain and weight as was observed between dose and gain. Thus the dependence of gain upon the size of the dose would

appear to be the fundamental consideration, although the constants included in the equations developed from figure 4 may be partially influenced by the increasing weight of the animal. Further studies of the relationship between size of dose, rate of gain, and weight of rat are now in progress, that sufficient data may be obtained for more accurate calculation of equations to determine vitamin B requirement.

The inferior response of the 0.1 gm. group must again be mentioned in view of the results obtained from figure 5. The amount of dose required for 1 gm. of gain was definitely more for this group than for either the 0.2, 0.3 or 0.5 gm. groups. It would seem that the 0.1 gm. level was too low and that the rat was unable to utilize the vitamin B of the wheat germ for growth because of other possible physiologic needs.

Length of the dose administration period. From the relation of dose to gain as plotted in figures 3 and 4 it would appear that the amount of gain is proportional to the amount of dose regardless of the length of the dose administration period. Valid estimates of the vitamin B content of a material may be obtained from short assay periods by merely calculating the amount of dose per unit of gain if rats of the same size have been used whose rates of gain were controlled within the limits of the 0.2 to 0.5 gm. groups, to eliminate the slight influence of weight.

TEN-DAY ASSAY PERIODS

The use of a 10-day dose administration period has been studied in a third series of rats. Eight groups of different sized rats, ranging in weight from 40 to 120 gm., were depleted of their vitamin B stores by means of ration B. When an animal was losing weight regularly, doses of the stabilized wheat germ were administered for a period of 10 days. The amount of dose to be given was determined each day from the gain of the rat for the previous day in order to keep the rate of gain within the limits of 1 to 2 gm. per day. This rate of gain was comparable to that of the 0.2 to 0.5 gm. groups of series II and was therefore directly proportional to the amount

of vitamin B in the dose. At the end of 10 days doses were discontinued, but the animal was weighed daily until it was again losing weight. If the rate of gain had been carefully controlled within the limits of 1 to 2 gm. per day, the rat usually started to lose weight the day after dose was stopped. Total dose was then divided by total gain to obtain a dose per unit gain figure.

The average amounts of dose required for a gram of gain for the different weight groups of series III were as follows:

WEIGHT GROUP	NUMBER OF RATS	TOTAL GAIN	TOTAL DOSE	AMOUNT OF DOSE FOR 1 GM. GAIN
42.0– 48.0	6	14.8	2.36	0.162[1]
51.0– 59.0	12	15.8	2.08	0.137
60.0– 64.5	17	14.8	2.05	0.144
65.5– 69.0	11	17.1	2.02	0.131
70.0– 77.0	5	14.8	2.07	0.140
80.0– 87.0	6	16.9	1.71	0.107
92.0– 99.0	6	16.9	2.83	0.142
100.0–135.0	4	18.0	2.40	0.135

[1] The animals of this smallest weight group seemed to become depleted more severely in relation to their losses of weight than rats in other groups.

The similarity in results for these averages for different groups would seem to indicate that valid results may be obtained from assay periods as short as 10 days when the rate of gain of the rat is controlled.

When the logarithms of the total dose and total gain for the 10-day assay groups were included in figure 4, they coincided with the straight line obtained from the figures for the 0.2, 0.3 and 0.5 gm. groups of series II, giving further proof that accumulative gain is dependent upon accumulative dose and is not related to the duration of the dose administration period. A relation between the weight of the rat and the amount of dose required for a unit gain was not apparent over the range studied since similar results were obtained for all weight groups. More irregularity, however, was encountered in the daily gains of the individual rats from the larger and smaller weight groups than for rats started at an initial weight of about 60 gm. Further experiments, particularly at higher

levels of weight, are now in progress to test more exactly the relations between animal weight and vitamin B requirement.

CONTROL EXPERIMENTS WITH CRYSTALLINE VITAMIN B

A series of twenty-one rats (initial weight 60 gm.) were given ration B until they were depleted of vitamin B stores. When the rats showed consistent losses of weight on consecutive days, doses of crystalline vitamin B[9] were given daily. The average response of the rats (fig. 6) was comparable to

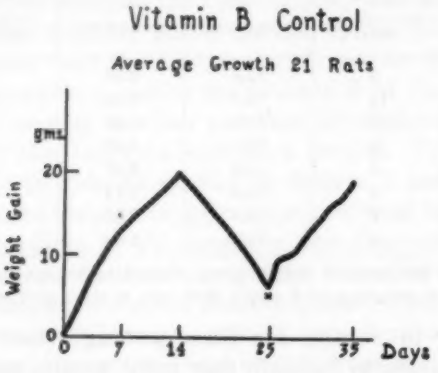

Figure 6

the growth obtained by Waterman and Ammerman ('35) at similar levels of intake since an average of 4 γ produced 1 gm. of gain.

SELECTION OF A UNIT FOR SHORT PERIOD ASSAYS

The 0.1 gm. group of series II (fig. 3) had a growth rate comparable to the 3 gm. per week recommended by Chase and Sherman as desirable for estimating 1 unit of vitamin B. The inferior response of this group, however, when growth

[9] Crystalline vitamin B was furnished through the courtesy of Merck & Co., Rahway, New Jersey.

was analyzed in relation to the amount of dose required for a gram of gain (as illustrated in figs. 3 and 5), may indicate that this growth rate is too near the borderline of requirement to be dependable as a unit. The consistent agreement between amount of dose and amount of gain for the 0.2, 0.3, and 0.5 gm. groups and for the 10-day assay groups when the rate of gain was kept within the limits of 1 to 2 gm. per day, would appear to justify the conclusion that a more satisfactory unit would be the amount of vitamin B producing 1 gm. of gain. This new unit is comparable in magnitude to the minimum curative dose described by Ammerman and Waterman ('35), and is approximately equal to two Chase and Sherman units.

SUMMARY

A short period technic for vitamin B assays has been developed through a study of the factors producing variability in results.

The amount of growth of the rat during 10 days was found to be a valid criterion of the quantity of vitamin B in the dose material if the following precautions had been observed.

1. The basal ration contained autoclaved whey in preference to autoclaved yeast, and sucrose instead of dextrin.
2. The rats weighed about 60 gm. when started on depletion.
3. The amount of dose was controlled each day to keep the rate of gain of the rats between 1 and 2 gm. per day.

The quantity of vitamin B causing 1 gm. of gain, when the rate of gain has been limited to 1 to 2 gm. per day, is suggested as a unit since it is comparable to the minimum curative dose defined by Ammerman and Waterman.

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A QUANTITATIVE STUDY OF THE UTILIZATION AND RETENTION OF VITAMIN B BY YOUNG CHILDREN [1, 2]

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TWO FIGURES

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Recent studies have indicated that an increase in vitamin B ingestion is frequently beneficial to infants and children. Gaynor and Dennett ('34) fed 100 normal infants a dried milk reinforced with a specially prepared water extract of rice polish as a source of vitamin B for an average period of 5 months. Fifty additional infants were fed an unfortified dried milk. In reference to the effect of the vitamin B extract, Gaynor and Dennett state:

The increase in weight was influenced favorably; metabolic efficiency was increased; anorexia and gastrointestinal disturbances were lacking; pallor was less marked; and nutrition was improved and a greater resistance to infection exhibited. They were as a group mentally more alert and less irritable, sleeping better and possessing practically none of the common complaints of infancy.

Morgan and Barry ('30) have reported favorable results when the vitamin B intake of under-weight children was increased. They obtained weight gains from 150 to 170% above

[1] The data of this study are taken from a dissertation submitted in partial fulfillment of the requirements for the degree of doctor of philosophy in nutrition, Child Welfare Research Station, State University of Iowa.

[2] The term vitamin B has been used for the fraction of the vitamin B complex which is sometimes designated vitamin B_1.

the expected gain when they fed two wheat germ rolls at the noon meal, while the control group which received white flour rolls gained only 50 to 71% of the expected gain during the same period.

Summerfeldt ('32) has likewise successfully applied vitamin B therapy to underweight children. Gains of 4.54 and 5.27 pounds in 10 weeks were produced by administering each week 4.2 ounces of wheat germ and 0.28 ounce of yeast, incorporated in a special breakfast cereal. The control groups, receiving ordinary cereals, gained only 1.25 and 1.27 pounds. That the gain with the special cereal was in part due directly to its vitamin B content has been shown by Ross and Summerfeldt in a more recent study ('35) where a third group received a vitamin B concentrate in addition to the ordinary cereal. The gain for this group was 5.5 pounds in 6 months as compared to gains of 3.6 pounds for the group receiving ordinary cereals and 7.8 pounds for the group receiving the special cereal.

If the prevalence of anorexia may be considered as an indication of inadequacy in regard to vitamin B, the following reports by Bartlett ('28) and Schlutz ('25) have particular significance in a study of children's requirements. After examination of 1471 consecutive records for children brought to the outpatient clinic, Bartlett concluded that anorexia far exceeded all other presenting symptoms in its incidence. Schlutz, in analyzing the role of the diet in the treatment of disorders of older infants and children, has said, "A complaint for which the child is brought to the physician more often than any other is persistent anorexia with cessation of gain in weight. . . . A problem is to provide abundant vitamin supply, particularly the water soluble vitamin B."

The evidence available, therefore, appears to indicate that dietary vitamin B for children frequently may be less than the amount required for optimum health. Thus a quantitative investigation of the utilization and retention of vitamin B by young children, as influenced by different levels of ingestion, seemed of value in determining requirements more exactly.

EXPERIMENTAL PROCEDURE

Children

The utilization of vitamin B has been studied through biological assay with rats of the amounts of vitamin B in the food and excreta of children receiving weighed diets. A total of twenty-three balance studies have been completed with eight children of 4 to 7 years of age. The children, who were selected from private homes and from a county juvenile home, were apparently in normal health. While being studied they were under constant supervision by nurses trained in metabolism technic.

Experimental period. The experimental period usually lasted 3 weeks during which time the same foods were served each day, the amount for each child being in proportion to his height and theoretical weight. To allow for adjustment to the level of vitamin B in the diet, the experimental period was divided, with one exception, into a 6-day preliminary period preceding two successive collection periods of either 4 or 5 days each. Extreme care was used to insure that the individually weighed portions of food were quantitatively consumed and the urine and feces quantitatively collected. The exception of the 6-day preliminary period was that related to the diet low in vitamin B. In this case, only a 1 day preliminary period was followed by 3 days of collections.

Children's diets. Three different levels of vitamin B ingestion have been tested. The diets varied chiefly in the selection of cereals and bread. For the highest and medium levels of vitamin B, whole wheat bread and cereal were contrasted with white bread and wheat endosperm cereal. For the lowest level the amount of vitamin B supplied by fruits, vegetables, and milk was decreased. The details of the various dietaries for D.G. and V.M. are given in table 1, since these children received dietary constituents comparable in kind and amount to those of the other children studied at each level of ingestion.[3]

[3] I am indebted to Miss Florence I. Scoular, research assistant, Iowa Child Welfare Research Station, for calculation of the diets and preparation of the meals.

Preparation of materials. All of the food for each collection period was prepared in advance for the entire period and stored in a low temperature refrigerator (8°C.), so that the servings for each day and the aliquot weighed for analysis

TABLE 1

Foods taken by the children during representative experimental periods (amounts in grams per day)

	11/23–25 D.G.	1/16–20 D.G.	2/25–3/1 D.G.	2/5–9 D.G.	11/14–17 D.G.	3/20–24 V.M.
Apple	80.0	139.0	85.0	35.0	139.0	159.0
Banana	50.0	126.0	41.0	31.0	126.0	126.0
Orange juice	15.0	61.5	62.7	62.6	125.0	127.2
Prunes (cooked)	....	20.1	20.1	20.1	20.1	26.4
Dates	10.0	....	16.0	....	....	....
Carrots	....	88.0	88.0	88.0	88.0	88.0
Tomatoes (cooked)	....	120.0	120.0	120.0	120.0	120.0
Onions	50.0	....	....	....	....	....
Potatoes	....	75.0	75.0	75.0	75.0	103.8
Rice (cooked)	60.0	....	....	....	....	....
Beef	60.0	60.0	60.0	60.0	60.0	36.0
Bacon	25.0	....	....	....	....	....
Egg	70.0[1]	115.0	110.0	80.0	115.0	75.0
Cheese (American)	30.0	....	....	....	....	....
Milk	305.0	488.0	488.0	976.0	488.0	488.0
Butter	35.0	33.0	31.0	19.0	32.0	32.0
Bread, white	60.0	48.0	....	....	....	....
Bread, whole wheat	....	....	25.0	26.0	72.0	70.0
Muffins (whole wheat)	....	....	33.0	....	....	....
Ralston	....	....	13.2	13.8	13.8	18.0
Cream of wheat	15.0	13.7	....	....	....	....
Tapioca	13.0	....	....	....	....	....
Sugar	44.0	23.0	15.0	15.0	24.0	19.0
Salt	1.0	1.0	1.0	1.0	1.0	1.0
Cod liver oil	12.0	12.0	12.0	12.0	12.0	12.0
Viosterol (drops)	8.0	8.0	8.0	8.0	8.0	8.0
Calories	1659.0	1615.0	1561.0	1540.0	1691.0	1650.0
Protein	53.5	57.3	62.6	64.9	59.8	51.0

[1] Egg white only.

might be identical in composition. Precautions, such as utilization of cooking water for vegetables, were followed to prevent, so far as possible, losses of vitamin B during the preparation of the foods. The food aliquots for the entire day to

be analyzed were mixed thoroughly with acid alcohol (95% ethyl alcohol plus 2% glacial acetic acid) and dried, first on a steam bath and finally to constant weight in an electric oven maintained at a temperature between 60° and 70°. Feces were preserved with acid alcohol and kept in a low temperature refrigerator (5°C.) until the amount for the entire period, as determined by carmine marking, was obtained. They were then pooled and dried in a manner similar to that used for food. Urine was made up to volume for each 24 hours and preserved under toluene in the refrigerator at 5°C. until pooled at the end of the collection period. It was then concentrated on a steam bath to one-twentieth of its original volume, the pH being adjusted to between 2.0 and 3.0 with hydrochloric acid.

BIOLOGICAL ASSAY

Young rats, weaned at about 28 days of age when their weight had reached 60 gm., were used for biological assay. These were placed in cages with raised screens ($\frac{1}{2}$ or $\frac{1}{3}$ inch mesh) and fed, ad libitum, the following basal depletion ration:

	gm.
Casein[4]	20.0
Autoclaved whey[5]	15.0
Cottonseed oil	20.0
Cane sugar	39.5
Cod liver oil	2.0
Salts—Wesson ('32)	3.5

The materials to be assayed were administered when the rats had commenced to lose weight regularly after 24 to 26 days of depletion. The dried food and feces were weighed on the analytical balance and fed directly, while the concen-

[4] Casein was freed from vitamins by soaking for 5 days in a 0.2% acetic acid solution changed twice daily, followed by three alternate treatments of solution in ammonium hydroxide and precipitation with acetic acid. After 48 hours of extraction in 80% alcohol, the first half hour being at 60°, the casein was dried at room temperature and ground.

[5] Dried whey (Peebles Lacto Milk) was obtained through the courtesy of the Western Condensing Co. of San Francisco. Four hundred grams of whey were mixed with an equal weight of distilled water, spread in a pan 9 × 15 inches, and autoclaved at its natural pH (5 to 6) for 2 hours at 18 pounds pressure.

trated urine was pipetted to tenths of a cubic centimeter and mixed with a little of the basal ration. Since the biological materials to be assayed were definitely limited in quantity, doses were administered to the rats for a period of 10 days. This method of assay was found to be valid when the size of the rat, length of the depletion period, size of the dose, and rate of gain of the rat were carefully controlled and standardized. The unit selected for comparison of results was the quantity of vitamin B causing 1 gm. of gain in the assay animals. This was calculated by dividing the total dose for 10 days by the total gain for that period. This unit was chosen because of its harmony with the minimum curative dose (Ammerman and Waterman, '35), the accuracy with which it may be calculated, and the simplicity with which it may be compared with a reference unit such as crystalline vitamin B. It is equal approximately to 2 Chase and Sherman ('31) units. The details of the assay technic have been given by Schlutz and Knott in the preceding paper.

DISCUSSION OF RESULTS

The detailed results of the assays for the various materials are given in table 2, at least three rats having been used to determine the vitamin B of each material. It is granted that this number of rats for an assay is less than the desired number, but the biological materials for this study were definitely limited in quantity. To offset this limitation, extreme care was used both in the technical details of the assay and in the evaluation of individual rat responses. All assays showing a too great degree of variability or a lack of harmony with other results obtained for the same level of vitamin B intake, were repeated to insure reliability of the final figures.

In assaying the amounts of vitamin B in the foods for the lower levels of intake (D.G., F.V., C.H. 11/23–25 and D.G., B.B. 1/16–20), difficulty was encountered in getting the rat to eat a large enough dose to furnish sufficient vitamin B to stimulate growth. It was necessary, therefore, to estimate from the assay data available the vitamin B content of these

foods. This procedure was considered to be more accurate than determination of the original food content by extracting the vitamin B, since such solvents as alcohol do not extract the vitamin quantitatively. The estimates were made on the basis of the amount of dose actually eaten by the rat, considering the amount which maintained weight or caused temporary growth as containing 1 unit of vitamin B. Since the weight of food containing 1 unit had to be (from the definition of a unit) greater than this largest amount of dose which was consumed without regular gain, the number of units as estimated for the daily food for D.G., F.V., and C.H. 11/23–25 and for D.G. 1/16–20 should be less than the amounts recorded in table 3.

The most significant result of the investigation was the definite trend toward higher retentions of vitamin B accompanying higher levels of intake in the children studied. These retentions have been plotted in figure 1 in relation to the level of vitamin B ingested. When the summary data for individual children, as presented in table 3, are considered it will be seen that retentions (calculated on the basis of the vitamin B of the food minus that of the urine), for the three children receiving different levels of vitamin B, increased with intake from 1 to 106 units for C.H., from 5 to 153 units for D.G., and from — 18 to 210 units for F.V. The total averages for each level of ingestion (table 4) also indicate that in spite of increasingly larger amounts of vitamin B in the urine as ingestion increased, the highest retentions, in all cases, occurred with the highest intakes. The average retentions of 147 and 169 units for the two highest ingestions of 224 and 311 units, respectively, are significantly greater than the average retentions of 12 and 27 units which accompanied the ingestions of 62 and 105 units. If the retentions based on the vitamin B of the food minus the total amount excreted in both urine and feces are considered the effect of the quantity of vitamin B in the diet is even more apparent, for retentions of 62 and 103 units were obtained with the higher levels of ingestion as compared to — 32 and — 14 for the two lower levels.

TABLE 2

Record of the individual rats used to determine the units of vitamin B in the food, urine and feces of the children studied

CHILD	DATE	ASSAY DATA FOR FOOD					ASSAY DATA FOR URINE					ASSAY DATA FOR FECES				
		Initial rat weight	Average dose per day	Gain of rat per day	Amount food for 1 unit vitamin B	Amount B per day	Initial rat weight	Average dose per day	Gain of rat per day	Amount urine for 1 unit vitamin B	Amount B per day	Initial rat weight	Average dose per day	Gain of rat per day	Amount feces for 1 unit vitamin B	Amount B per day
		gm.	*gm.*	*gm.*	*gm.*	*units*	*gm.*	*cc.*	*gm.*	*cc.*	*units*	*gm.*	*gm.*	*gm.*	*gm.*	*units*
C.H.	11/23–25	56.5	4.0[1]	....	....		63.5	1.00	1.46	0.57	61	68.5	0.43	1.87	0.19	63
		60.5	4.0[1]	....	....		67.0	0.98	1.60	0.61	57	84.5	0.28	1.45	0.19	63
		78.5	4.0[1]	....	....							79.0	0.33	0.90	0.37	33
		73.5	5.0[1]	....	....											
C.H.	11/14–17	64.0	2.2	1.40	1.57	208	90.5	0.65	2.08	0.31	129	60.0	0.77	0.95	0.81	33
		73.5	1.9	1.10	1.68	195	57.0	0.63	2.41	0.24	166	65.5	0.62	1.50	0.38	69
							69.5	0.52	1.36	0.35	115	63.0	0.64	1.05	0.61	43
C.H.	11/18–21	64.0	2.2	1.40	1.57	208	66.5	0.60	1.50	0.40	100	58.0	0.59	0.85	0.69	31
		73.5	1.9	1.10	1.68	195	63.5	0.55	1.14	0.44	91	64.5	0.40	1.55	0.26	81
												78.0	0.42	0.65	0.65	33
B.B.	1/16–20	68.5	3.7	0.50	4.7	64	65.0	1.19	1.17	1.1	45	68.5	0.60	1.40	0.43	36
		54.5	3.4[1]	....	....		73.5	1.31	1.00	1.3	38	72.0	0.56	1.15	0.49	31
		59.0	4.0[1]	....	....		69.0	1.82	1.43	0.9	56	70.5	0.69	1.45	0.48	32
		66.0	4.5[1]	....	....											
D.G.	2/25–3/1	53.0	2.9	0.80	3.63	81	55.5	0.66	0.78	0.85	71	53.5	0.55	1.22	0.45	36
		65.5	3.2	0.83	3.80	77	63.0	1.17	1.12	1.04	58	70.5	0.57	1.17	0.49	34
												56.0	0.49	1.16	0.38	43
		62.0	3.1	1.00	3.54	83										
		64.0	2.4	1.00	2.4	122										
D.G.	11/23–25	59.0	3.5[1]	....	....		66.5	0.74	1.30	0.57	61	71.0	0.66	1.55	0.43	21
		69.5	3.4[1]	....	....		53.5	0.76	1.15	0.66	53	66.0	0.54	1.30	0.42	21
		74.0	4.0[1]	....	....		70.0	1.21	1.95	0.69	51					
D.G.	1/16–20	76.0	3.0[1]	....	....		63.5	0.53	0.88	0.6	83	69.0	0.51	1.55	0.33	47
		65.5	5.0[1]	....	....		57.5	1.00	1.35	0.7	71	64.5	0.51	1.21	0.39	40
		51.5	4.0[1]	....	....		68.5	1.40	1.35	1.0	50	73.0	0.42	1.06	0.40	39
							68.0	1.10	1.20	0.9	56					
D.G.	2/ 5– 9	71.5	2.6	1.25	2.2	133	62.5	1.05	1.15	0.9	67	68.5	0.86	1.61	0.48	39
		69.0	3.2	1.35	2.3	127	66.5	1.20	1.83	0.7	86	67.0	0.71	1.40	0.51	37
D.G.	11/14–17	60.0	2.1	1.32	1.57	221	60.5	0.98	1.35	0.56	89	74.0	0.21	1.00	0.21	102
		64.0	2.1	1.35	1.54	225	66.5	1.18	1.75	0.67	75	77.0	0.30	1.45	0.21	102
D.G.	11/18–21	60.0	2.1	1.32	1.57	221	63.0	1.08	1.32	0.74	68	60.0	0.42	1.70	0.25	82
		64.0	2.1	1.35	1.54	225	69.0	0.95	1.35	0.70	71	82.0	0.31	1.41	0.22	93
J.F.	2/25–3/1	62.0	3.4	1.35	2.48	116	60.5	1.07	1.25	0.86	70	73.0	0.46	1.15	0.34	41
		64.0	3.0	1.00	2.54	113	67.0	1.27	1.94	0.91	66	70.5	0.45	1.40	0.32	45
		73.5	1.6	0.61	2.60	111										

D.G.	11/18–21	60.0	2.1	1.32	1.57	221	63.0	1.08	1.32	0.74	68	60.0	0.42	1.70	0.25	82
		64.0	2.1	1.35	1.54	225	69.0	0.95	1.35	0.70	71	82.0	0.31	1.41	0.22	93
J.F.	2/23–3/1	62.0	3.4	1.35	2.48	116	80.5	1.07	1.25	0.86	70	73.0	0.40	1.15	0.34	41
		64.0	3.0	1.00	2.54	113	67.0	1.27	1.04	0.91	66	70.5	0.45	1.40	0.32	43
		73.5	1.6	0.61	2.60	111										
F.V.	11/23–25	61.0	3.0[1]	....	....		66.0	1.33	1.13	1.18	30	84.0	0.42	1.30	0.32	51
		73.5	3.0[1]	....	....		73.5	1.21	0.90	1.21	29	68.0	0.36	1.22	0.29	56
		52.5	3.0[1]	....	....											
		66.5	5.0[1]	....	....											
F.V.	1/16–20	69.0	2.5	0.50	4.7	63	67.5	0.84	0.79	1.0	50	67.0	0.65	1.23	0.48	41
		65.0	3.5	1.25	4.8	62	66.5	0.97	0.89	1.1	45	82.5	0.28	0.96	0.29	68
		67.0	4.5	....			78.0	0.98	0.80	1.2	42	75.5	0.34	1.00	0.34	58
F.V.	12/10–14	68.0	2.7	0.75	3.5	97	71.0	0.76	1.20	0.6	83	61.0	0.54	1.95	0.28	85
		57.0	3.7	0.79	3.8	89	59.0	0.68	1.40	0.5	100	65.5	0.56	1.23	0.24	99
		61.0	4.5	....												
F.V.	2/ 5– 9	72.0	2.6	0.80	3.3	85	67.0	0.96	1.27	0.7	86	78.0	0.62	1.50	0.41	47
		70.5	3.5	0.94	3.7	76	87.0	1.03	1.78	0.6	100	74.5	0.77	1.45	0.53	37
		70.0	4.0	1.30	3.1	90	65.5	0.74	1.45	0.5	120	82.0	0.57	1.25	0.46	42
F.V.	11/14–17	63.0	1.6	1.08	1.39	244	67.0	1.00	0.75	1.13	44	63.5	0.41	1.25	0.33	66
		70.0	1.9	1.35	1.37	247	59.5	1.35	1.00	1.34	37	77.0	0.25	1.65	0.15	146
												70.5	0.28	1.15	0.24	91
F.V.	11/18–21	63.0	1.6	1.08	1.39	244	60.0	0.76	2.11	0.36	37	62.5	0.53	1.50	0.27	91
		70.0	1.9	1.35	1.37	247	66.0	0.75	1.95	0.38	36	62.5	0.31	1.32	0.21	117
							61.0	0.64	1.60	0.40	36	57.0	0.22	1.40	0.16	154
J.S.	3/15–19	56.0	2.3	2.11	1.08	324	65.5	0.76	1.91	0.40	125	52.0	0.47	1.85	0.25	97
		68.0	1.9	1.75	1.05	333	66.0	0.64	1.45	0.40	125	62.0	0.58	1.80	0.32	76
J.S.	3/20–24	80.0	2.5	1.46	1.53	229						57.0	0.22	1.70	0.13	180
		68.0	1.7	1.35	1.22	287	54.0	0.66	1.71	0.37	138	69.0	0.31	1.70	0.18	130
		73.0	2.0	1.67	1.21	289										
		57.5	2.2	1.41	1.60	219	58.5	0.48	1.40	0.34	150					
P.D.	3/15–19	Not assayed See results of similar food for period 3/20–24					72.0	0.73	1.50	0.49	104	72.0	0.83	1.63	0.51	53
												69.5	0.91	1.40	0.65	42
P.D.	3/20–24	68.0	1.7	1.41	1.18	275	80.0	0.69	1.59	0.44	123					
		73.5	2.1	1.95	0.95	341	54.5	0.62	1.77	0.32	168	67.0	0.53	0.85	0.62	43
							67.5	0.45	1.30	0.35	154	71.0	0.49	1.15	0.43	61
V.M.	3/15–19	65.0	1.9	2.10	0.91	373	70.0	0.59	1.33	0.44	125	58.5	0.66	2.70	0.24	92
							60.5	0.74	1.65	0.45	122	62.0	0.42	2.15	0.20	110
V.M.	3/20–24	67.0	1.8	2.46	0.74	459	65.0	0.56	1.95	0.29	190	74.5	0.57	1.70	0.34	59
		62.0	2.1	2.40	0.88	386	70.0	0.49	1.64	0.27	204	66.5	0.30	1.50	0.20	100
							74.5	0.47	1.67	0.24	229	70.0	0.36	1.75	0.23	87

[1] For explanation of these periods see text, paragraph 2 of 'Discussion of Results.'

Both of the methods of calculating vitamin B retentions (food minus urine and food minus total excretion) have been employed in this study since our knowledge of the metabolism of vitamin B is too limited at the present time to permit a

TABLE 3

The vitamin B intakes, excretions and retentions of the individual children for each period, in units of vitamin B per day

CHILD	DATE	AGE	WEIGHT	VITAMIN B				
				Food	Urine	Feces	Retention	
							Food-urine	Food-total excretion
		yr.—mo.	*kg.*	*units*	*units*	*units*	*units*	*units*
C.H.	11/23–25	4– 9	16.3	60[1]	59	53	1	— 52
C.H.	11/14–17	4– 9	15.8	202	137	48	65	17
C.H.	11/18–21	4– 9	15.8	202	96	48	106	58
B.B.	1/16–20	4– 2	15.4	64	46	33	18	— 15
D.G.	11/23–25	4– 8	19.3	60[1]	55	21	5	— 16
D.G.	1/16–20	4–10	19.8	63[1]	65	42	— 2	— 44
D.G.	2/25–3/1	4–11	19.9	91	65	38	26	— 12
D.G.	2/ 5– 9	4–10	19.8	130	77	38	53	15
D.G.	11/14–17	4– 8	19.3	223	82	102	141	39
D.G.	11/18–21	4– 8	19.3	223	70	89	153	64
J.F.	2/25–3/1	4– 2	19.0	113	68	42	45	3
F.V.	11/23–25	3–11	16.1	60[1]	30	54	30	— 24
F.V.	1/16–20	4– 1	16.4	63	46	56	17	— 39
F.V.	2/ 5– 9	4– 2	16.8	84	102	42	— 18	— 60
F.V.	12/10–14	4– 0	16.6	93	92	92	1	— 91
F.V.	11/14–17	3–11	15.9	246	41	101	205	104
F.V.	11/18–21	3–11	15.9	246	36	121	210	89
J.S.	3/15–19	6– 9	21.5	328	125	87	203	116
J.S.	3/20–24	6– 9	21.5	256	144	155	112	— 43
P.D.	3/15–19	4– 8	15.8	308	104	48	204	156
P.D.	3/20–24	4– 8	15.8	308	148	52	160	108
V.M.	3/15–19	5– 3	21.1	373	124	101	249	148
V.M.	3/20–24	5– 3	21.1	422	208	82	214	132

[1] Estimated.

complete explanation of the significance of vitamin B in the urine and feces. The vitamin B excreted in the urine cannot be entirely a surplus of intake above requirement since vitamin B is present in appreciable amounts in urine during low levels of ingestion and furthermore the amount in the urine

does not increase comparably to food increases when the level of ingestion is raised. The vitamin B of the feces may be partially unabsorbed residues from the food, but similarly to the findings with urine, the quantity in the feces does not increase proportionately to increases in food intake. In addition, it is apparent that a relation between the excretion

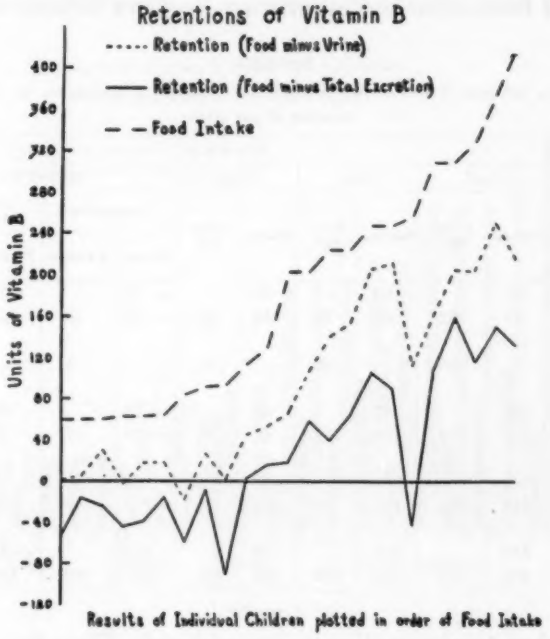

Figure 1

of vitamin B in the feces and the urine may exist since there was a trend in this study for feces figures to be high when urine was low and vice versa. Further work is now in progress to learn more regarding vitamin B metabolism and an attempt is being made to study the factors influencing the excretion of vitamin B in urine and feces.

Gaynor and Dennett ('34) have reported that their data for infants indicate but little storage of vitamin B in the body. A similar conclusion may be reached from analysis of the retentions for D.G., F.V., and C.H., 11/23–25. Although high ingestion periods (11/14–17, 11/18–21) preceded the period 11/23–25 by only one day, the three children showed an average retention of only 12 units when calculated on the basis of food-urine and an average negative balance of — 31

TABLE 4

Average vitamin B values for each level of vitamin B ingestion, in units of vitamin B per day

NUMBER OF CHILDREN	VITAMIN B									
	Food		Urine		Feces		RETENTION			
							Food-urine		Food-total excretion	
	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average
6	60		30		21		— 2		— 52	
	64	62	65	50	56	43	— 30	12	— 15	— 32
1		93		92		92		1		— 91
4	84		65		38		— 18		— 60	
	130	105	102	78	42	40	— 53	27	15	— 14
6	202		36		48		65		17	
	246	224	137	77	121	68	210	147	104	62
6	256		104		48		112		— 43	
	422	311	208	142	155	88	249	169	156	103

units when total excretion was considered. Thus the average total retention of 62 units for the preceding period was not sufficient to counteract the low ingestion of period 11/23–25. It would seem, therefore, that excess supplies of vitamin B are quickly exhausted from the body.

In view of this apparent inability of the body to build up a reserve of vitamin B, the need for an adequate supply is particularly important. As an aid in determining how much is required for an optimum intake, the vitamin B contents of food, urine and feces have been calculated on a per kilogram

basis for each child and plotted, in order of amount ingested, in figure 2. It will be noted that ingestions were not consistently above the total quantity of vitamin B excreted until more than 12 units per kilogram were included in the daily allowance. The highest retentions were obtained with an ingestion of 20 units of vitamin B per kilogram. Since retentions consistently continued to increase with increased ingestions, the tentative conclusion is advanced that at least 20 units

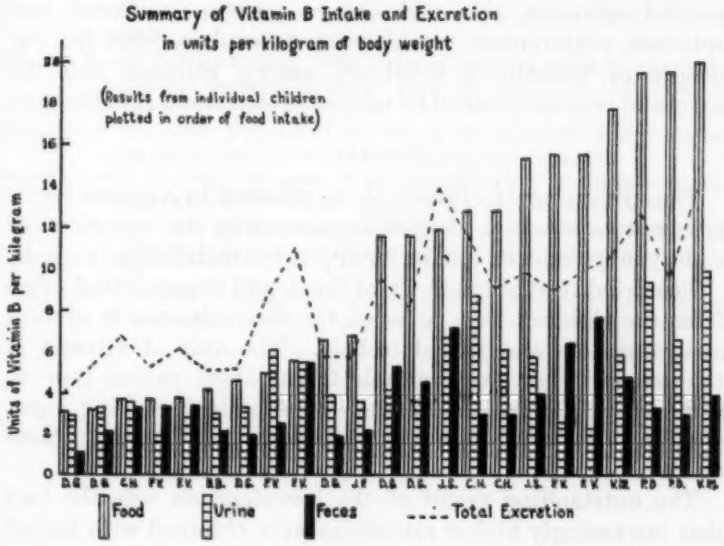

Figure 2

of vitamin B per kilogram of body weight are necessary each day as a satisfactory intake to insure optimum health. In terms of Chase-Sherman units, this intake is approximately equivalent to 40 units per kilogram per day.

This high vitamin B requirement is comparable to the recent findings of Waterman and Ammerman ('35) with purified vitamin B. These authors state: "It should be noted that the upper limit of beneficial increment of the vitamin has not been reached although the vitamin intake which can generally

be secured by a choice of natural foods has apparently been surpassed.'' In the present study, the intakes which resulted in the highest retentions (regardless of whether retentions were calculated on the basis of food minus urine or food minus total excretion) were found to be six to seven times greater than the minimum requirement for preventing beri-beri as determined by means of the formula suggested by Cowgill ('34).

If the intakes resulting in highest retentions may be considered optimum, this wide range between minimum and optimum requirement would seem to explain both the existence of vitamin B deficiency among children and the beneficial results obtained by additions of vitamin B to the diet.

SUMMARY

The utilization of vitamin B, as affected by various levels of ingestion has been studied by comparing the retentions of eight young children during twenty-three metabolism periods.

The dried food aliquots, dried feces, and concentrated urine from the children were assayed for their vitamin B content according to standardized technic. The unit of vitamin B selected as being most suitable to the short period type of assay employed was the quantity of vitamin B causing 1 gm. of gain. This unit is approximately equal to 2 Chase-Sherman units.

The outstanding result of the investigation was the fact that increasingly higher retentions were obtained with higher intakes for each level of ingestion studied.

On the basis of the ingestions giving the highest retentions, the optimum requirement of vitamin B by young children is estimated to be 20 units per kilogram of body weight, or about 40 Chase-Sherman units per kilogram per day.

The writer wishes to take this opportunity to thank Dr. Amy L. Daniels, whose help and advice made this study possible, and also to thank members of the nutrition staff of the Iowa Child Welfare Research Station for their cooperation.

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THE SPECIFIC DYNAMIC ACTION OF BUTTER FAT, AND OF SUPERIMPOSED SUGAR

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SIX FIGURES

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In a previous paper from this laboratory by Hawley, Johnson and Murlin ('33) on the possibility of gluconeogenesis from fat in the human subject, some data relative to the specific dynamic effect of the high fat (cream) meals were presented. A more complete treatment of this phase of high fat metabolism was reserved for study with the new respiration calorimeter (Murlin and Burton, '35). The purpose of using the calorimeter for this study was to obtain, if possible, reliable measurements of the heat value of a liter of oxygen at respiratory quotients below the level for combustion of fat by making use of the direct heat. It was believed that heat values found by extrapolation could be fairly checked by that means. This would make possible the construction of dynamic curves from which the total effect of a given meal could be deduced, even though the R.Q. should fall considerably below 0.707. The interpretation of these low R.Q.'s should be more secure once the heat relations were understood.

EXPERIMENTAL

All the work has been done on nine male subjects, all but one of them members of the department as staff or students.

They ranged in age from 23 to 61 years and in weight from 68 to 94 kg. Several of the experiments represent the subject's first experience on high fat and several exhibit the signs of improved tolerance on repetition of the diet which were discussed in the earlier report.

The calorimeter was supplemented in the indirect determination of heat production by the Tissot-Haldane method. Having a particularly efficient Haldane air analyzer, designed by Dr. E. S. Nasset, it has not been unduly burdensome either to observer or subject to superimpose the short 10-minute periods with the Tissot, thereby completing the record without keeping the subject confined too long. In the tables following, *T* just before the time of period stands for the Tissot-Haldane and *C* for the calorimeter. It has been gratifying to find that the two totally different types of determination of indirect heat often agreed, where they were supposed to agree, quite as well as could be expected (e.g., table 6, June 1; table 7, June 22). Candor requires the further statement, however, that in some instances (e.g., table 1, January 16) the agreement is not too good. Such differences are attributable to variations in temperature or to the discomfort sometimes caused by a nose clip.

In some of the later experiments, for convenience in attaching the surface thermometers (Burton, '34, '35) the subject entered the calorimeter nude except for a pair of 'shorts,' and yet the basal in this condition, when the temperature was equally comfortable, duplicated the basal taken by the spirometer method when the subject was fully clothed (e.g., table 6, June 1). When there was a difference of as much as 3 or 4° between room temperature and calorimeter temperature, the agreement was not so good. However, with a subject possessing a liberal amount of subcutaneous fat this difference in temperature did not increase the metabolism (table 4, June 13) when he was nude.

Check experiments

Numerous heat checks have been run during the period, from June 1933 to July, 1936, within which these experiments were made, in order to be assured that the calorimeter was operating correctly. On recalibration for heat loss through the wall (Murlin and Burton, '35) after the summer vacation of 1934 a slightly different calibration curve was obtained and for the last six experiments, done since that time, the new curve has been used.

Altogether forty-five alcohol check periods were run from May, 1933 to June, 1936, at times adjacent to the experiments. The average R.Q. obtained was 0.663 with an average deviation from the mean of $\pm$ 0.018. The average difference between direct and indirect heat in individual periods was 3.6%,[1] the algebraic mean + 1.69% or 1.3 Cal. The direct heat was the higher. The agreement between different respiratory quotients from the same expired air with the Tissot-Haldane method is better than $\pm$ 0.01.

Calculation of indirect heat for R.Q.'s below 0.707

While accumulating data on the heat value of a liter of oxygen for low R.Q.'s by the direct measurement, it was necessary meantime to compare direct and indirect heats for evaluation of the specific dynamic action. Inspection of the low quotients in the tables reveals that in comparison with other periods with higher quotients, they are due in most instances to high oxygen values rather than to low CO_2 values. This is best seen in basal periods on the Tissot (e.g., tables 1 and 2). It has been customary in many laboratories to calculate heat production for non-protein R.Q.'s less than 0.707 as if the quotient were 0.707, thereby admitting error in the determination and closing the mind to the possibility of uses of oxygen other than for combustion. While it is true that in the closed circuit method there are more chances of error on oxygen than on CO_2, with frequent alcohol checks it is not necessary

[1] Exactly the same whether calculated on CO_2 or O_2.

to concede serious error (see check experiments). With the growing evidence that O_2 may be used for other purposes, it is conservative to regard the CO_2 as correct and to base the calculation of indirect heat on the assumption that all of the non-protein CO_2 represents combustion of fat in these low quotients. If the CO_2 is correct and the extra O_2 which is responsible for the low R.Q. is used for some other purpose than combustion, the heat production on the basis of CO_2 at an R.Q. of 0.707 and the heat production on the basis of O_2 at the value $(\frac{4.686 \times \text{low R.Q.}}{0.707})$ will be the same. This is shown in the footnote.[2]

Either method of calculation obviously gives a lower value than to base the calculations on the oxygen at the non-protein R.Q. of 0.707. In other words, the assumption is made that the extra oxygen which gives the low R.Q. is being used entirely, as we know from the ketosis that it is being used in part, for some purpose other than complete combustion. Furthermore, this procedure gives better agreement with the direct measurement in most cases,[2] as Adams and Poulton[3] ('36) have shown is true on the basis of the data of DuBois. Having adopted this method of calculation for low quotients obtained with the calorimeter, it was necessary to adopt it also for the Tissot periods, even though the oxygen determinations with this method are just as reliable as the CO_2.

A few comparisons are possible between the heat values for a liter of oxygen obtained in this way with the values

[2] For example the following comparisons may be cited:

	Indirect heat calculated on			
Non-protein R.Q.	1 O_2 at non-protein R.Q. of 0.707 Cal.	2 CO_2 at non-protein R.Q. of 0.707 Cal.	*Extrapolation* $\frac{4.686\ Cal. \times low\ N.P.\ R.Q.}{0.707\ Cal.}$	*Direct heat*
0.60	107.2	93.7	94.3	84.6
0.61	99.2	87.1	87.0	91.5
0.61	100.0	92.0	91.8	96.8
0.65	80.8	78.7	78.7	76.0
0.68	88.8	85.1	85.2	78.3
0.689	78.3	74.5	74.5	74.5
0.695	100.1	98.7	98.6	98.0

[3] Our data had been assembled and tabulated as above before we were aware of the work of Adams and Poulton.

obtained from direct heat, by subtracting protein calories and dividing by the liters of non-protein oxygen. Thus,

	Heat value of a liter of O_2	
Non-prot. R.Q.	4.686 × low R.Q.	Direct heat—prot.Cal.
	0.707	Non-prot. O_2
0.60	3.976 Cal.	3.521 Cal.
0.61	4.043	4.281
0.64	4.241	4.355
0.65	4.308	4.355
0.675	4.292	4.473
0.68	4.507	4.277
0.686	4.546	4.566
0.69	4.573	4.572

There is not yet in the collection of data a sufficient number of calorimeter periods with low R.Q.'s and determination of the nitrogen in the urine to extend the comparison further. The agreement between the two methods depends upon the errors of direct heat measurement and those implicit in the protein heat. With perfect measurement and evaluation of these two factors oxygen values necessarily agree.

The mean indirect heat for the total of forty-two periods with these subjects is 83.01 ± 0.85 Cal. and for the direct heat 83.31 ± 0.88 Cal. per hour, a difference of only 0.3 Cal. or 0.36% of the indirect.[4] The real test of agreement in any particular period, however, is the mean of the individual differences, which is ± 3.28 Cal. or 3.95%, practically the same as the alcohol checks. Only one other series of heat measurements on human subjects employing both direct and indirect calorimetry in hourly periods ever has been reported in which the percentage difference between the two means was less than the 0.36% recorded here (Gephart and DuBois, '15).

[4] The significance ratio as to the differences of the means is found by comparing the difference, 0.30 Cal., with its probable error. Unless it is three times the probable error of the difference the difference itself is usually regarded as insignificant. The P.E. of the difference of means is found by the formula $\sqrt{E_1^2 + E_2^2}$ (Davenport, '04) where E_1 and E_2 are the probable errors of the two means, 83.01 and 83.31. The formula $0.6745\frac{\sigma}{\sqrt{n}}$ gives E. The standard deviations are 8.17 and 8.51 Cal., respectively. Therefore the P.E. of the difference of the means is 1.22 and the ratio 0.25; which is to say that in two such series of measurements of the same quantity, a difference of 0.3 Cal. shows that one method (direct) is just as dependable as the other (indirect).

The authors reported a difference between their totals for 61 hours of 0.17%. Their series, however, contains only fifty-four periods[5] in which figures are correctly given for both indirect and direct measurements on the hourly basis. The difference of the means in these fifty-four periods is 0.25 Cal. or 0.324% of indirect. The mean difference between the two methods is ± 4.49 Cal. or 5.8% of indirect. It is evident that the present series, shorter by ten periods, shows a slightly better agreement in the average hourly determination than that of Gephart and DuBois, notwithstanding their slightly better agreement between totals.

It is gratifying to find that the agreement between direct and indirect heat improves a little as more experience with the calorimeter is gained. In describing the calorimeter (Murlin and Burton, '35) it was stated (p. 258) that the average percentage difference in individual periods was of the order of 7 or 8%, but that by use of the surface thermometers together with the rectal to indicate change of body temperature, the difference had been brought down to ± 5.5%. None of the first four experiments of the present series was among those in which the improved method of securing change of body temperature was adopted. With five of the later subjects (V to IX inclusive) the surface temperatures were measured; but in one (VII) because of an accident to the chest thermometer, they could not be used. In only 5 of the calorimeter days out of the 18 here reported therefore was any use made of the surface temperatures. Rectal temperature, of course, was used in all. It is hoped that still better agreement may be attained by use of the surface thermometers in future work. The improvement in precision, however, is due mainly to the use of CO_2 as a basis for calculation of the indirect heat for low R.Q.'s as described above.

[5] There are several obvious errors of addition in their table 3. These are excluded as well as the results given in two hourly determinations.

PROCEDURE

Certain features of the daily program on experimental days will be apparent from the representative tables. Following in a general way the dietary procedure of a former study (Hawley, Johnson and Murlin, '33) for the highest fat intake (F.A. to G ratio of 4.1), heavy (4X) cream in amount necessary to cover the 24-hour caloric requirements was taken. The only adjuvants permitted were lettuce and coffee, and the lettuce was generally omitted on calorimeter days. It served the purpose mainly of affording something to masticate and to cleanse the mouth of the creamy after-taste. The coffee, sweetened occasionally with saccharine, afforded a vehicle for more agreeable ingestion of a large part of the cream. The lettuce rarely exceeded 100 gm. at a meal and the coffee two cups. As a rule the subject ingested the all-cream diet for a total period of at least 5 days. Probably no other diet supplying practically all the energy from fat can be tolerated equally well for so long a period. According to the earlier experience the lowest R.Q.'s were obtained only after the second day. Exploratory determinations therefore were made on the third day of the diet, with the Tissot-Haldane method, and in several instances the calorimeter was used to get a basal determination. The fourth day of the diet was expected to be the most favorable for low quotients, but it did not turn out so in all cases. After further exploratory determinations in post-absorptive conditions on this day, the subject then took a large meal of fat (the largest he could tolerate) and entered the respiration calorimeter for the double determination of the heat production for several hours. Some twenty subjects have been studied in the calorimeter to date and all but two or three have found the bed and surroundings comfortable for a total of 3 to 4 hours. Practically complete muscular repose can be maintained.

CALCULATION OF S. D. A.

Specific dynamic action of the food, as the term is used in this paper, refers to the increase in heat production over post-absorptive basal. Of the several known ways of expressing the specific dynamic effect of food, that one originated by Benedict and Carpenter ('18) which includes the entire area between the base line and the curve of excess and expression in terms of calories fed seems to the present writers to be the most suitable for study of ordinary foodstuffs. Where food substances of known molecular weight in crystalline purity can be given, doubtless the expression in terms of mols (Wilhelmj, '34) is to be preferred. Percentage increase over basal is also given in the individual tables.

Construction of the complete curve of excess over basal must of necessity be a piecemeal procedure for a calorimeter. Even if the subject could endure a continuous experiment for 10 successive hours and maintain complete repose, the observers would find it exceedingly laborious. By starting at different intervals after feeding meat and running a few hours each day, Williams, Riche and Lusk ('12) were able to follow its dynamic effect in the dog to completion, and the same method was used by Murlin and Lusk ('15) for the dynamic action of fat in the dog. The purpose of the present study made it impossible to follow this method systematically, but a sufficient number of points on the curve was obtained to draw a plausibly complete curve for all but one subject. The curves will be described in the next section.

RESULTS

It will not be possible, because of limitation of space, to present all the results except in summary. Three experiments on as many subjects taking high fat alone were done in June and July, 1933 when the weather was quite hot. Subject I had been on the all-cream diet previously in 1930 and 1931, but it may be supposed that any special adaptation to high fat acquired at that time had been lost in the 2 years and 5 months intervening. It is also possible that the hot weather made

the fat diet less tolerable, in the sense of less perfect combustion, for all three subjects. Subject II had not had previ ous experience with the diet; he exhibited a very low tolerance. Subject III had taken it for 5 days 2 weeks earlier in July and had exhibited many more low respiratory quotients than he did in the later experiment. There is evidence of adaptation therefore in his case (Hawley, Johnson and Murlin, '33). The subject who had lost any special adaptation to the metabolism of high fat showed quite low quotients in the basal periods as well as in the calorimeter.

The heavy cream used (38 to 41% ether extract) contained all but about 5% of the calories from fat (Sherman, '33). Subjects I and II ingested as a test meal 450 gm. with a small amount of lettuce which would supply not more than 5 gm. carbohydrate. The specific dynamic action of the 171 gm. fat in the case of subject I reached on the first day the level of 20.3% above basal at about the third hour (time always calculated to middle of the respiratory or calorimetric period). The next day at about ½ hour earlier, it rose to 24.6%. These figures compare favorably with the value of 23% obtained by Murlin and Lusk ('15) in a dog after ingestion of 75 gm. emulsified peanut and lard oils mixed. Subject II in his highest period gained a level of only 16.2%, which probably is explained by poor digestion. In fact this subject was so distressed by the high fat that he was obliged to discontinue the experiment the next morning after the first basal period. Subject III endured the diet very well after the previous adjustment period 2 weeks earlier, and on the fifth day produced a dynamic effect from 152 gm. fat which at the high point was 27% above basal. The basal periods, however, were obtained the day before. Eighty grams fat with this subject in corresponding periods the first day gave a dynamic effect a little more than half as great as after 152 gm. the second day. To turn the comparison about, a dose 1.9 times as great produced a dynamic effect 1.8 times as great as the first day. Digestion and absorption obviously were satisfactory.

Three experiments were done in January, 1934 (illustrated by tables 1 and 2) in which two important modifications from the previous experiments were made. First, after the calorimetric study on the fourth day of the high fat diet, a large dose of sugar was taken and the study of dynamic effect was continued by the Tissot-Haldane procedure. The same dose was repeated after the basal periods on the fifth day. R.Q.'s and S.D.A. of the superimposed sugar could now be compared in the two conditions. Subjects I and II were the same persons of corresponding numbers as in the earlier experiments. Probably because of the time of year and almost certainly because both subjects 6 months before had become at least partially adapted (II) and readapted (I), respectively, they tolerated the diet much better. At all events they metabolized the fat more completely, as judged by the R.Q.'s. Secondly, also the ketosis was followed by analysis of the blood and urine for ketone bodies. Subject I following a somewhat larger meal of fat, had shown a ketosis (table 3) of approximately the same order in 1931 (Hawley, Johnson and Murlin, '33, p. 535) and R.Q.'s within the same range.

Subject IV was having his first experience with high fat (table 2). He was also the youngest of the subjects and a rather stout, but not quite obese, person. Although the test meal of fat taken on the fourth day of the diet was decidedly smaller than that taken by subject I, the resulting ketosis of subject IV was considerably higher (table 3). Also the basal ketosis was the highest on each of the three days (third, fourth and fifth of the diet) shown by any of the subjects. We are prepared therefore for lower R.Q.'s in this subject. He had all the signs except alimentary distress of low tolerance for fat. In the second calorimeter period, the non-protein R.Q. reached the low point of 0.61, which is far too low to be accounted for by any degree of ketosis and possible formation of sugar from glycerol and protein (Hawley, Johnson and Murlin, '33, p. 554). It should be observed also that the basal R.Q.'s by the Tissot-Haldane method on the third and fourth days of the diet were quite low.

TABLE 1

Specific dynamic action of butter fat and of sucrose superimposed (subject II)

DATE 1934 JANUARY	TIME[5] AFTER SUGAR	TIME[5] AFTER FAT MEAL	APPLIANCE	Period	CO_2	O_2	R.Q.	N./HR.	NON-PROTEIN R.Q.	HEAT CAL./HR.		S.D.A. PER CENT OF INDIRECT
										Indirect	Direct	
	minutes	*minutes*			*L./hr.*	*L./hr.*		*gm.*				
16[1]		Basal	T	8.50– 9.00 A.M.	11.37	15.92	0.71	0.432	0.70	73.2[2]		
		Basal	T	9.08– 9.18 A.M.	11.04	15.53	0.71	0.432	0.69	71.0[2]		
				In calorimeter—no food								
			C	11.15–12.16 P.M.	11.66	16.03	0.73	0.412	0.71	74.6	78.5	
17			T	8.53– 9.03 A.M.	10.85	14.85	0.72	0.490	0.71	69.0		
			T	9.09– 9.19 A.M.	10.95	13.90	0.79	0.490	0.78	65.5		
				9.40 Took 240 gm. 4X cream = 91 gm. fat.								
				In calorimeter								
		91	C	10.51–11.52 A.M.	13.15	17.41	0.75	0.508	0.74	81.6	83.0	21.4[3]
		161	C	11.52–12.51 P.M.	12.72	16.89	0.75	0.508	0.74	79.1	76.3	13.5
										160.7	159.3	
				1.23 Took 100 gm. sucrose								
	52	275	T	2.10– 2.20 P.M.	15.29	18.38	0.83	0.487	0.84	88.0		30.8
	85	308	T	2.43– 2.53 P.M.	15.21	17.18	0.88	0.487	0.90	83.3		24.1
	130	353	T	3.28– 3.38 P.M.	13.6	16.22	0.84	0.487	0.85	77.7		15.6
18		Basal	T	8.49– 8.59 A.M.	9.96	15.33	0.65	0.234	0.63	64.9[4]		
		Basal	T	9.06– 9.16 A.M.	10.51	14.11	0.74	0.234	0.74	65.6		
				9.45 A.M. Took 100 gm. sucrose								
	53		T	10.33–10.43 A.M.	14.07	16.34	0.86	0.399	0.87	78.8		20.1[4]
	83		T	11.03–11.13 A.M.	14.56	17.00	0.86	0.399	0.86	82.0		25.0
	130		T	11.50–12.00 M	13.80	16.46	0.84	0.399	0.84	78.9		20.2

[1] Third day of all-cream diet.

[2] Calculated on non-protein R.Q. found, by extrapolation from 0.707.

[3] Using 67.2 Cal./hr. mean of indirect as basal.

[4] Using 65.6 Cal./hr. mean of indirect as basal.

[5] To middle of experimental period.

T = Tissot-Haldane method; C = calorimeter.

TABLE 2

Specific dynamic action of butter fat and of glucose superimposed (subject IV)

DATE 1934	TIME[5] AFTER SUGAR	TIME[5] AFTER FAT MEAL	APPLIANCE	RESPIRATORY EXCHANGE					HEAT CAL./HR.		S.D.A. PER CENT OF INDIRECT
				Period	CO_2	O_2	R.Q.	N./hr.	Non-protein R.Q.	Indirect	Direct	
	minutes	*minutes*			*L./hr.*	*L./hr.*						
January		Basal	T	8.52– 9.02 A.M.	12.87	18.99	0.68	0.506	0.66	83.4[3]		
30[1]		Basal	T	9.14– 9.24 A.M.	12.43	17.62	0.70	0.506	0.69	80.1		
31		Basal	T	8.52– 9.02 A.M.	12.86	18.02	0.71	0.518	0.69	82.9[3]		
		Basal	T	9.13– 9.23 A.M.	13.12	18.90	0.69	0.581	0.67	84.0		
				10.05 A.M.	Took 250 gm. 4X cream = 95 gm. fat							
				10.30 A.M.	In calorimeter							
		91	C	11.12–12.00 A.M.	13.90	19.88	0.70	0.672	0.67	88.6[3]	96.3	6.1[3]
		140	C	12.00–12.49 P.M.	14.40	22.30	0.65	0.672	0.61	93.0[3]	96.8	10.1
				1.30 P.M.	Took 100 gm. glucose							
	52	259	T	2.19– 2.29 P.M.	15.87	21.51	0.74	0.858	0.72	99.9		19.7
	87	292	T	2.52– 3.02 P.M.	16.02	21.38	0.75	0.858	0.74	99.7		19.5
	147	352	T	3.52– 4.03 P.M.	14.57	18.89	0.73	0.858	0.708	92.1		10.3
February 1		Basal	T	8.46– 8.56 A.M.	12.37	17.17	0.72	0.519	0.702	79.8		
		Basal	T	9.08– 9.18 A.M.	12.33	16.95	0.72	0.519	0.71	78.7		
				9.45 A.M.	Took 100 gm. glucose							
	54		T	10.34–10.44 A.M.	14.80	19.20	0.77	0.673	0.76	90.1		13.6[4]
	93		T	11.13–11.23 A.M.	15.00	18.72	0.80	0.673	0.80	88.5		11.7
	148		T	12.08–12.18 P.M.	15.42	18.54	0.83	0.673	0.84	88.4		11.5

[1] Third day of all-cream diet.

[3] Calculated on heat value of non-protein R.Q. found, by extrapolation from 0.707.

[3] Using 83.5 Cal./hr. mean of indirect as basal.

[4] Using 79.3 Cal./hr. mean of indirect as basal.

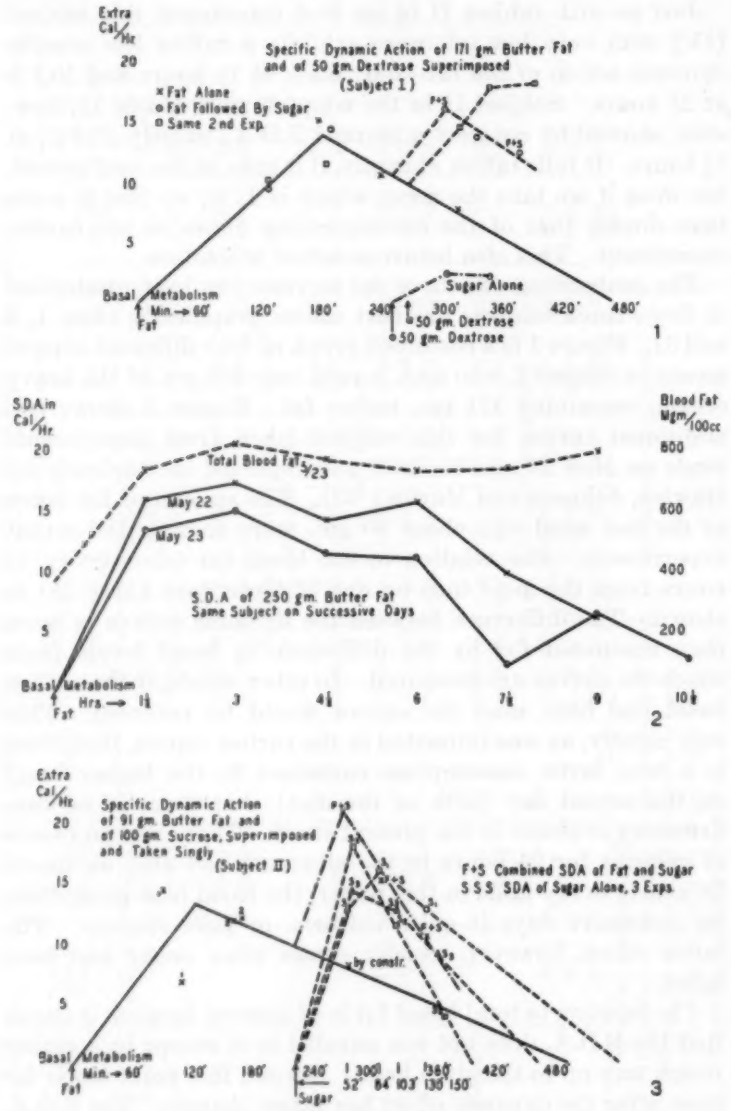
[5] To middle of experimental period.

T = Tissot-Haldane method; C = calorimeter.

Just as with subject II in his first experiment this subject (IV) with very low tolerance exhibits a rather low specific dynamic action of the fat—only 6.1% at 1½ hours and 10.1% at 2½ hours. Subject II in the adapted state (table 1), however, showed by contrast a marked S.D.A., namely 21.4%, at 1½ hours. It falls rather abruptly, it is true, in the next period, but even if we take the mean which is 17%, we find it more than double that of the corresponding figure in his earlier experiment. This also betokens better utilization.

The contrasting effects of fat to raise the heat production in these three subjects are best shown graphically (figs. 1, 2 and 3). Figure 1 is a combined graph of four different experiments on subject I, who took in each case 450 gm. of the heavy cream, containing 171 gm. butter fat. Figure 2 shows two additional curves for this subject taken from experiments made on May 22 and 23, 1930 and reported incompletely by Hawley, Johnson and Murlin ('33). The amount of fat taken at the test meal was about 80 gm. more than in the recent experiments. The relation to the blood fat taken every 1½ hours from the meal time on one of these days (May 23) is shown. The difference between the dynamic curves is more than accounted for by the difference in basal levels from which the curves are measured. In other words, if the earlier basal had been used the curves would be reversed. This may signify, as was intimated in the earlier report, that there is a true luxus consumption contained in the higher basal on the second day (fifth of the diet); but there is no confirmatory evidence in the present study. There was no excess of calories for 24 hours in the all-cream diet and, as shown by nearly every table in this paper, the basal heat production on successive days is quite uniform, or falls slightly. The latter effect, however, usually comes after sugar had been taken.

The relation to total blood fat is of interest because it shows that the S.D.A. does not run parallel to it except in a rather rough way up to the sixth hour. Beyond this point blood fat rises after the dynamic effect has fallen sharply. The S.D.A.



Figures 1 to 3

of fat therefore does not appear to be due entirely to plethora in the sense of Lusk.

All the S.D.A. curves show that the rise is much more abrupt than the decline, and there is a sort of family resemblance in the general proportions of all of them.

Figure 1 is the best authenticated curve, for it is a composite of four experiments all of which gave points which agreed well. Figure 3, on the contrary, is averaged graphically from two experiments which were not very concordant and one point on the down slope was obtained 'by construction,' i.e., by subtracting the average effect of three sugar experiments from the effect of fat plus the same amount of sugar in one. The resulting curve is too high (table 8) and is included only for the sake of showing the comparison between the superimposed sugar curve and the three curves after sugar alone, which is discussed below.

The third group of experiments carried out in May and June, 1935, were identical in procedure with the preceding group. All the subjects, V, VI and VII-'35 were young graduate students, one 25 and the other two 24 years of age. Subjects V and VI were slender, subject VII quite stout, almost obese. None of them had had previous experience with high fat diets, but all exhibited higher tolerance than the three preceding subjects. This may be indicated by the following brief table of data borrowed from the succeeding article.

TABLE 3

Indicated tolerance for fat in basal period on fourth day of all-cream diet

SUBJECT	KETONEMIA	KETONURIA
	mg./100 cc.	*mg./hr.*
I	31.9	308
II	50.9	237
IV	55.3	264
V	21.0	38
VI	20.9	71
VII-'35	31.4	95
VII-'36	14.2	25
VIII	36.4	176
IX[1]	25.8	210

[1] Fifth day.

As previously emphasized by Hawley, Johnson and Murlin ('33) there is no very close correlation between the respiratory quotient and ketonemia or ketonuria, but a low figure in both these indices combined with high or 'normal' R.Q.'s denotes high tolerance; high figures for ketosis and low for R.Q.'s, low tolerance.

These subjects, accordingly showed very few low quotients, the lowest (0.666, non-protein 0.64) occurring as a basal period with subject V. Subject VI gave no non-protein R.Q.'s below 0.70 and subject VII-'35 only one (0.69 in the third calorimeter period on June 14th; table 4). The response to the high fat meal, which consisted of 400 gm. (or slightly more) of the heavy cream in all three cases, was an average non-protein R.Q., for the three full-hour calorimeter periods, of 0.74, 0.74 and 0.72. The specific dynamic effect was an increased heat production which averaged for the same three periods, respectively, 15.4, 21.4 and 23.1% above basal. Table 4 illustrates this group of experiments.

The S.D.A. curves for subjects V, VI and VII are given in figures 4, 5 and 6. The biphasic appearance of two of these may be due to the almost uncontrolable somnolence which is produced by the high fat metabolism at about the end of the third hour, or to periodicity in the evacuation of fat from the stomach, or both. One subject certainly slept a few minutes in this middle period and the others were not quite sure but that they had fallen asleep momentarily. The 'anaesthetic effects' of diacetic acid have been established by Peterman ('24) and commented upon from this laboratory elsewhere (Marsh and Murlin, '28). The low point in the middle period is followed by a high point the next, which may be accounted for by a fresh evacuation of the cream to the intestine by the stomach. Subject VII-'35 was certainly a little more restless in the calorimeter than the other two.

The last three experiments were performed in June 1936. Subject VII was the same individual who bears this number in the 1935 series. The two experiments are distinguished by the year date, thus (VII-'35 and VII-'36). Because he was

TABLE 4

Specific dynamic action of butter fat and of sucrose superimposed (subject VII–'35)

DATE 1934 JUNE	TIME[1] AFTER SUGAR	TIME[1] AFTER FAT MEAL	APPLIANCE	RESPIRATORY EXCHANGE					HEAT CAL./HR.		S.D.A. PER CENT OF INDIRECT
				Period	CO_2	O_2	R.Q	N/hr.	NON-PROTEIN R.Q.	Indirect	Direct	
	minutes	*minutes*			*L./hr.*	*L./hr.*		*gm.*				
13		Basal	T	9.36– 9.46 A.M.	13.95	17.30	0.81	0.334	0.81	82.6		
				10.40	In calorimeter—no food—nude							
			C	11.47–12.48 P.M.	12.55	16.32	0.77	0.297	0.76	77.0	81.1	
			C	12.48– 1.42 P.M.	12.94	16.79	0.77	0.297	0.77	79.4	82.0	
14		Basal	T	8.59– 9.09 A.M.	13.65	17.47	0.78	0.376	0.78	82.7		
		Basal	T	9.25– 9.35 A.M.	13.37	17.55	0.76	0.376	0.75	82.6		
				9.45 A.M.	Took 400 gm. 4X cream = 152 gm. fat							
				10.00 A.M.	In calorimeter—false start							
		147	C	11.42–12.42 P.M.	16.48	22.46	0.73	0.520	0.72	104.9	101.3	26.8[4]
		207	C	12.42– 1.42 P.M.	14.98	19.62	0.76	0.520	0.75	92.3[2]	101.7[3]	23.1
		268	C	1.42– 2.44 P.M.	15.29	21.51	0.71	0.520	0.69	98.6[2]	98.0	19.3
										295.8	301.0	
				3.13 P.M.	Took 25 gm. sucrose in 300 cc. water							
	61	389	T	4.09– 4.19 P.M.	15.34	20.84	0.73	0.454	0.73	97.5		18.0
	126	454	T	5.14– 5.24 P.M.	15.51	18.31	0.84	0.454	0.85	88.0		6.4
	182	510	T	6.10– 6.20 P.M.	15.89	18.09	0.87	0.454	0.89	87.6		6.1
				6.55 P.M.	Took 400 gm. 4X cream + 100 gm. lettuce							
15				3.05 A.M.	Took 25 gm. sucrose in 300 cc. water							
		Basal	T	8.48– 8.58 A.M.	12.93	16.77	0.77	0.307	0.77	79.4		
		Basal	T	9.17– 9.27 A.M.	12.73	17.18	0.74	0.307	0.73	80.5		
				9.55 A.M.	Took 25 gm. sucrose in 300 cc. water							
	74		T	11.04–11.14 A.M.	13.71	18.34	0.74	0.321	0.74	86.2		7.8[5]
	133		T	12.03–12.13 P.M.	13.43	17.14	0.78	0.321	0.78	81.3		1.6
	191		T	1.01– 1.11 P.M.	13.88	17.50	0.79	0.321	0.79	83.2		4.1

[1] Subject slept few minutes.

[2] Calculated on heat value of non-protein R.Q. found, by extrapolation.

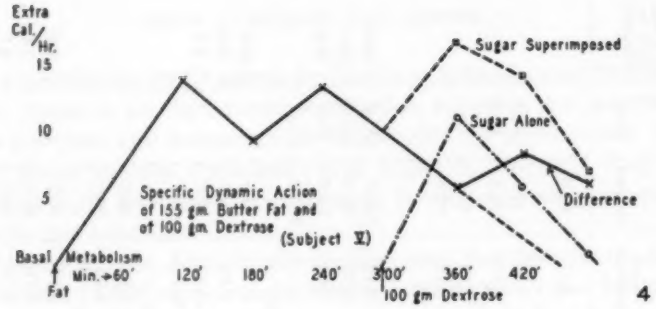
[3] Direct heat used for calculation of S.D.A. this hour.

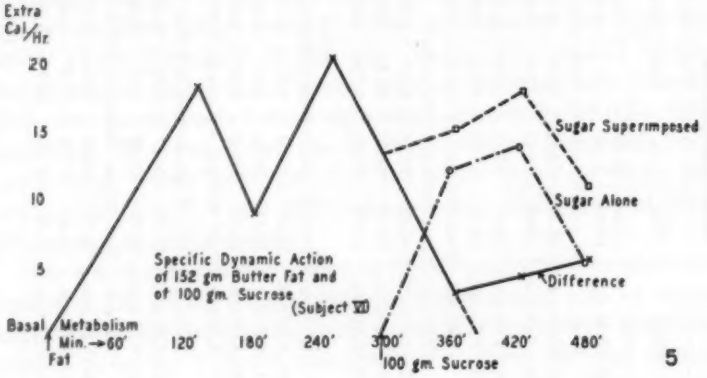
[4] Using 82.6 Cal./hr. mean of indirect as basal.

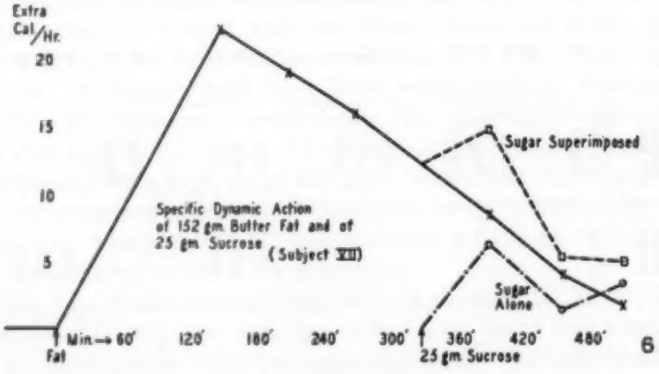
[5] Using 79.9 Cal./hr. mean of indirect as basal.

[6] To middle of experimental period.

T = Tissot-Haldane method; C = calorimeter.







Figures 4 to 6

rather uncomfortable in the calorimeter the entire series of respiratory determinations with him in 1936 was carried out by means of the Tissot-Haldane procedure. In the 1935 experiment he had exhibited rather high tolerance for the cream diet; the ketosis was low (table 3) and the R.Q.'s high. In the 1936 experiment, however, he had considerable stomach distress causing hyperpnoea and as a consequence gave two periods with excessively high R.Q.'s after taking 350 gm. of the cream (table 5). The R.Q. for the third period of this test is probably correct, for the symptoms of gastric distress and hyperpnoea had entirely passed off. The dynamic effect of this meal is comparable with that of the corresponding meal in 1935 (table 4, June 14th) obtained with the calorimeter. Two of the periods in each case center at the same intervals after taking food. The sum of the extra heats calculated to 1 hour in each case is 41.4 Cal. for the meal of 152 gm. fat in 1935 and 40.4 Cal. for the meal of 144 gm. fat in 1936—very nearly an exact proportion. Slight discomfort in the calorimeter in the first test was matched by the over breathing in the second. Both give dynamic effects above the average (table 8).

The next day (fifth of high fat) in the 1936 experiment shows several abnormally low R.Q.'s both in the early morning and later morning periods. Two of these occur after 25 gm. glucose had been given, clearly demonstrating that a similar dose of sugar the previous day had not restored the glycogen of the liver sufficiently to permit combustion (see following paper). To the same effect is the logical inference from the low quotients earlier in the day. Also the ketosis by this time had risen considerably. The combined dynamic effect of sugar and fat, however, is larger on this than on the preceding day (table 5).

Subject VIII had the highest ketosis on the fourth day of diet of the last group (table 3). Accordingly, in the basal determination in the calorimeter on June 1st (table 6) he shows one abnormally low R.Q. and one also after taking the meal of 330 gm. 4X cream. The dynamic effect of the meal is low as

TABLE 5

Specific dynamic action of butter fat and of superimposed sugar (glucose) subject VII-'36

DATE 1936 JUNE	TIME[2] AFTER SUGAR	TIME[2] AFTER FAT	APPLIANCE	RESPIRATORY EXCHANGE				NITROGEN IN URINE	Non-Protein R.Q.	HEAT CAL./HR. INDIRECT	S.D.A. EXTRA CAL./HR. OVER BASAL	PER CENT OF INDIRECT BASAL
				Period	CO_2	O_2	R.Q.					
	minutes	*minutes*			*L./hr.*	*L./hr.*		*gm./hr.*				
8[1]	Basal		T	8.31– 8.41 A.M.	12.85	17.25	0.75	0.396	0.74	80.85	Average	
	Basal		T	9.15– 9.25 A.M.	13.06	17.45	0.75	0.396	0.74	81.87	basal	
				8.36 A.M.	350 gm. 4X cream (144 gm. fat)						81.36	
		85	T	10.56–11.06 A.M.	17.48	20.11	0.89	0.483	0.88	97.29	15.93[4]	19.6
		145	T	11.56–12.06 P.M.	17.87	20.86	0.86	0.483	0.86	100.64	19.28[4]	23.7
		205	T	12.56– 1.06 P.M.	16.43	21.81	0.75	0.483	0.75	102.49	21.13[5]	24.9
		224		1.20 P.M.	25 gm. glucose in 300 cc. water							
	65	289	T	2.20– 2.30 P.M.	15.83	20.35	0.78	0.560	0.77	95.99	14.63	18.0
	125	349	T	3.20– 3.30 P.M.	14.51	20.02	0.72	0.560	0.71	93.10	11.74	14.4
	185	409	T	4.20– 4.30 P.M.	13.72	19.10	0.72	0.560	0.70	88.74	7.38	9.1
9				6.22 A.M.	350 gm. 4X cream (144 gm. fat)							
		138	T	8.35– 8.45 A.M.	14.01	19.73	0.71	0.613	0.69[3]	89.72	8.36	10.2
		178	T	9.15– 9.25 A.M.	15.55	21.29	0.73	0.613	0.71	99.13	17.77	21.8
		195		9.35 A.M.	25 gm. glucose in 300 cc. water							
	69	262	T	10.39–10.49 A.M.	15.34	21.50	0.71	0.600	0.70[3]	98.66	17.30	21.3
	127	320	T	11.37–11.47 A.M.	15.45	21.63	0.72	0.600	0.70	99.36	18.0	22.1
	185	378	T	12.35–12.45 P.M.	15.79	21.31	0.74	0.600	0.73	99.57	18.21	22.4

[1] Fourth day of all-cream diet.

[2] Calculated to middle of experimental period.

[3] Heat value of oxygen by extrapolation from R.Q. 0.707.

[4] Excessive breathing caused by stomach distress, produced high R.Q.'s.

[5] Breathing normal.

TABLE 6

Specific dynamic action of butter fat and of superimposed sugar (fructose) subject VIII

DATE 1936 JUNE	TIME[2] AFTER SUGAR	TIME[2] AFTER FAT	APPLIANCE	RESPIRATORY EXCHANGE					NITROGEN IN URINE	HEAT CAL./HR.		S.D.A. EXTRA CAL./HR. OVER INDIRECT	PER CENT INDIRECT
				Period	CO_2	O_2	R.Q.	NON-PROTEIN R.Q.		Indirect	Direct		
	minutes	*minutes*			*L./hr.*	*L./hr.*			*gm./hr.*				
1[1]	Basal		T	8.41– 8.51 A.M.	13.26	16.92	0.78	0.78	0.504	79.94			
	Basal		T	9.06– 9.16 A.M.	11.93	15.99	0.75	0.73	0.504	74.69			
				9.40 A.M.	In calorimeter, nude								
	Basal		C	11.04–12.09 P.M.	11.77	16.60	0.71	0.69[3]	0.550	75.2	76.1[4]	Average	
	Basal		C	12.09– 1.09 P.M.	12.30	16.75	0.73	0.72	0.550	77.9	77.9	basal	
				1.40 P.M.	330 gm. 4X cream (132 gm. fat)							76.93	
				2.00 P.M.	In calorimeter, nude								
		104	C	2.54– 3.54 P.M.	11.62	16.38	0.71	0.69[3]	0.501	74.5	74.5		
		164	C	3.54– 4.54 P.M.	13.44	18.12	0.74	0.73	0.501	84.7	79.8	7.77	10.1
		224	C	4.54– 5.54 P.M.	13.51	17.80	0.76	0.75	0.501	83.5	83.13	6.63	8.6
		275		6.15 P.M.	50 gm. C.P. fructose in 300 cc. water								
	70	345	T	7.20– 7.30 P.M.	14.58	18.87	0.77	0.77	0.442	89.13		12.20	15.8
	140	415	T	8.30– 8.39 P.M.	16.64	18.59	0.89	0.91	0.442	80.55		13.62	17.7
	198	473	T	9.30– 9.37 P.M.	20.02	17.99	1.11	1.16	0.442	92.07		15.14	19.6
		318		4.00 A.M.	330 gm. 4X cream (132 gm. fat)								
2		287	T	8.44– 8.50 A.M.	10.51	18.36	1.06	1.09	0.268	93.32		16.39	21.4
				9.18 A.M.	50 gm. C.P. fructose in 300 cc. water								
	68	386	T	10.23–10.29 A.M.	20.61	18.46	1.12	1.15	0.283	94.89		17.96	24.4
	129	447	T	11.24–11.30 A.M.	19.40	16.73	1.16	1.20	0.283	86.73		9.80	12.7
	189	507	T	12.23–12.28 P.M.	22.06	17.78	1.25	1.29	0.283	93.77		16.84	21.9

[1] Fourth day of all-cream diet.

[2] To middle of experimental period.

[3] Heat value of oxygen by extrapolation.

[4] Using rectal temperature only for this period.

was observed with subject II (p. 621), and with subject IV (p. 624 in table 2) under similar conditions. The ketosis in subject VIII, however, was not quite so high and the R.Q.'s not so low as with subject IV. The low R.Q.'s while not directly proportional to the ketosis, evidently are related to poor combustion and therefore to the specific dynamic effect of the fat, for subject VIII gave no dynamic effect the first calorimeter hour after the fat meal (really the second hour after the meal) when the R.Q. was lowest, and quite low figures even when the R.Q.'s were normal. Respiration was normal in the calorimeter.

This subject's respiratory center, however, became very sensitive to ketosis later, for in the early morning periods on June 2nd, his respiration rate rose to 30 per minute. The second early morning period (which is shown in table 6) it had fallen to 24 per minute, but the R.Q. is still abnormally high. To some extent a high respiration rate is responsible for the high quotient following the ingestion of C.P. fructose the day before; but the highest quotient accompanied the lowest rate and therefore can scarcely be due to the acidosis alone. Carpenter and Fox ('30) have shown that doses of levulose as small as 5 gm. perceptibly raise the R.Q. and Carpenter and Lee ('33) have shown that the rise in quotient after 25 gm. fructose is not due to the formation of organic acids causing diminished alveolar CO_2. Quotients higher than unity so often obtained with this sugar, without any acidosis Carpenter believes, are only to be explained by the formation of fat (see also Deuel, '27).

In the present instance we cannot with confidence explain the high respiratory quotients as due to fat formation because of the known ketosis and the signs of respiratory stimulation.[6] The sugar was given to determine its specific dynamic effect when superimposed on fat, to compare this combined effect with that of other sugars, and also to observe its effect on the ketogenesis (see following paper). With full reservation as to the correct explanation of the high quotients, the results on dynamic action are shown in table 6. The high quotient in

[6] See table 7, following article, for effect on Co_2-combining power of the blood.

the early morning period on June 2nd, 4¾ hours after fat, must be due in large measure to the ketosis causing a high respiration rate. The dynamic effect of fat at this time, however, calculated on the high non-protein R.Q. with allowance for fat formation is no higher than is seen in many other subjects (table 8). Further comment on the effect of sugar will be found in the following sections.

Subject IX developed ketosis on the high fat rather more slowly than any other subject (table 3). His tolerance for fat from every other observable criterion was good, yet he showed a number of low R.Q.'s by both methods, some of them even after sugar was ingested. Table 7 shows many other interesting details. The specific dynamic action of 123 gm. fat with not over 6% of the calories from other food stuffs is calculated from a curve constructed from only the five periods after fat on June 23rd and 24th. The average coincides with the grand average shown in table 8.

A summary of the specific dynamic action of butter fat in the nine subjects is assembled in table 8. The extra calories per hour are derived from S.D.A. curves, as shown in the representative figures, by dropping perpendiculars from the curve to the base line at the middle point of the successive hours spanned by the curve. The summation of these successive hourly levels is substantially equivalent to the area under the curve. The two experiments on subject I at the end of the table taken from data published previously, are introduced for comparison because there was an actual determination of the heat production at intervals of 1½ hours all through the day up to 9 hours after the meal in one and 10½ hours in the other. These two curves from complete records give final values which are within the same range as the others in the table. The summation of total S.D.A. calories, expressed as a percentage of the fat calories contained in the test meal, shows a range for the subjects able to maintain a perfectly satisfactory state of repose of 3.55 to 6.0 with a mean value of 4.74%. The corresponding value given by Murlin and Lusk for dogs ('15) is 4.1%. Subjects II and VII-'35 are excluded

TABLE 7

Specific dynamic action of butter fat and of superimposed sugar (sucrose) subject IX

DATE 1936 JUNE	TIME[2] AFTER SUGAR	TIME[2] AFTER FAT	AP-PLIANCE	RESPIRATORY EXCHANGE				NITROGEN IN URINE	NON-PROTEIN R.Q.	HEAT CAL./HR.		S.D.A.	
				Period	CO_2	O_2	R.Q.			Indirect	Direct	Extra Cal./hr. over indirect	Per cent of indirect
	minutes	*minutes*			*L./hr.*	*L./hr.*		*gm./hr.*					
22[1]	Basal		T	8.25– 8.35 A.M.	11.93	16.25	0.73	0.773	0.70	75.1			
	Basal		T	9.02– 9.12 A.M.	12.31	16.33	0.75	0.773	0.72	75.7			
				9.50 A.M.	In calorimeter, nude								
	Basal		C	11.04–12.10 P.M.	11.62	15.73	0.74	0.756	0.72	74.8	75.7	Average	
			C	12.10– 1.04 P.M.	12.35	16.51	0.75	0.756	0.72	76.6	73.6	basal	
				1.22 P.M.	300 gm. 4X cream (123 gm. fat)							75.19	
		245	T	5.22– 5.32 P.M.	12.89	18.37	0.70	0.721	0.67[3]	81.77		6.58	8.7
		305	T	6.22– 6.32 P.M.	13.56	18.31	0.74	0.721	0.72	85.08		9.89	12.0
23	Basal		T	8.32– 8.42 A.M.	12.19	16.45	0.74	0.634	0.72	76.43		Average	
			T	9.12– 9.22 A.M.	12.18	16.41	0.74	0.634	0.72	76.26		basal	
				9.47 A.M.	300 gm. 4X cream (123 gm. fat)							76.34	
				10.45 A.M.	In calorimeter, nude								
		142	C	11.33–12.42 P.M.	13.74	19.77	0.70	0.631	0.67[3]	87.85	....[4]	11.55	15.2
		205	C	12.42– 1.45 P.M.	13.75	19.67	0.70	0.631	0.67[3]	87.84	85.06	11.60	15.3
		262	C	1.45– 2.33 P.M.	14.47	19.67	0.74	0.631	0.72	91.58	90.12	15.24	20.0
		303		2.50 P.M.	50 gm. sucrose in 300 cc. water								
	66	369	T	3.51– 4.01 P.M.	12.99	18.58	0.70	0.595	0.67[3]	83.09		6.75	8.8
	127	430	T	4.52– 5.02 P.M.	13.27	18.19	0.73	0.595	0.71	84.52		8.18	10.7
	187	490	T	5.52– 6.02 P.M.	12.02	16.37	0.73	0.595	0.71	76.06		0.0	0.0
24				4.55 A.M.	300 gm. 4X cream (123 gm. fat)								
		247	T	8.57– 9.07 A.M.	13.87	18.25	0.76	0.417	0.75	85.76		9.42	12.2
		292	T	9.42– 9.52 A.M.	13.64	18.63	0.73	0.417	0.72	86.99		10.65	14.0
		306		10.01 A.M.	50 gm. sucrose in 300 cc. water								
	67	373	T	11.03–11.13 A.M.	14.72	19.47	0.75	0.426	0.74	91.57		15.23	19.0
	125	431	T	12.01–12.11 P.M.	14.39	19.37	0.74	0.426	0.73	90.67		14.33	20.0
	186	492	T	1.02– 1.12 P.M.	11.32	15.80	0.71	0.426	0.70	73.57		0.0	0.0

[1] Fourth day of all-cream diet.

[2] Calculated to middle of experimental period.

[3] Heat calculated by extrapolation to this R.Q.

[4] Obvious large error on direct heat.

[2] Fourth day of all-cream diet.
[3] Calculated to middle of experimental period.
[4] Heat calculated by extrapolation to this R.Q.
[5] Obvious large error on direct heat.

TABLE 8

Specific dynamic action of butter fat expressed as a percentage of the calories fed

SUBJECT	AGE	WEIGHT	EXTRA CALORIES PER HOUR										NUMBER HOURS AVERAGE	EXTRA CALORIE SUMMATION	FAT CALORIES IN FOOD	TOTAL S.D.A.	AVERAGE EXTRA CAL./HR.
			1	2	3	4	5	6	7	8	9	10					
		kg.														%	
I	60	94	2.5	6.8	11.4	12.5	9.7	6.8	3.8	1.0			7.8	54.5	1534	3.55	7.0
II	31	75	4.5	14.3	12.3	10.2	8.0	6.0	3.8	1.7			8.2	60.8	819	(7.42)[2]	7.4
III	45	68	2.2	6.6	12.7	16.7	16.6	11.6	6.7	1.5			7.8	74.6	1368	5.45	9.6
IV	23	88	1.7	5.1	9.2	7.2	6.0	2.5					5.8	31.7	855	3.70	5.4
V	25	73	3.5	10.7	11.2	11.2	11.3	7.7	3.3	0.0			7.5	58.9	1395	4.22	7.8
VI	24	70	4.0	12.9	15.2	15.0	18.0	8.5	0.0				6.4	73.6	1368	5.38	11.5
VII-'35	24	92	4.5	13.6	22.1	19.0	15.8	12.2	8.5	4.3	1.7		8.5	101.7	1368	(7.43)[3]	12.0
VII-'36	25	92	2.5	7.2	11.8	16.5	17.0	12.7	8.5	4.2			8.0	80.4	1339	6.0	10.0
VIII	25	71	1.7	5.1	8.6	12.0	16.0	12.7	7.7	2.5			8.0	66.3	1227	5.4	8.3
IX	27	73	2.0	6.1	10.2	14.2	15.0	6.0	0				6.0	53.5	1144	4.7	8.9
I[1]	56	93	5.2	15.8	16.7	16.2	14.1	15.0	13.6	9.7	7.3	2.0	10.0	115.6	2250	5.13	11.5
I[1]	56	93	4.3	13.3	14.3	13.7	11.4	11.1	8.0	2.1	5.0	2.8	10.0	86.0	2250	3.8	8.6
											Average		7.0	71.4	2024	4.74	9.0

[1] Taken from paper by Hawley, Johnson and Murlin ('33).
[2] Subject somewhat restless; not used in average.

from this average because the former was considerably upset by the diet on one of his experiments and the latter was somewhat 'fidgety' in the calorimeter. The lowest dynamic effects by this index were shown by the oldest subject and by the youngest, who as it happens was quite corpulent and not at all inclined to muscular exercise. Subject VII was equally corpulent but was at the same time quite active muscularly. Aside from the subjects II and VII-'35 the next highest score was made by subject III, a man who lives by manual labor, and subject VI, who was well-developed muscularly and kept in training by playing squash frequently.

THE S.D.A. OF SUPERIMPOSED SUGAR

This is well shown by the curves in figures 1, 3, 4, 5 and 6. There are two types of response: 1) fairly complete summation; and 2) greater effect when sugar is superimposed than when taken alone. The first is illustrated by figures 3 and 6 and is recognized by the fact that the curve for fat (continuous line) falls at a uniform rate, where, toward the end, the values for sugar alone are subtracted from the curve obtained by superimposition. The second type is illustrated by all the other curves showing effects of sugar (figs. 1, 4 and 5). There were no separate determinations of the dynamic effect of sugar alone for the last three subjects. The first type is the one which would be expected if there is no interference with absorption of either food when mixed in the alimentary tract. Murlin and Lusk ('15) obtained nearly complete summation of the separate dynamic effects of fat and glucose and of these two with glycocoll in the dog. Type 2 is the more interesting because it seems to denote a more favorable utilization of the fat when sugar is superimposed upon it. As shown most strikingly in figure 4, the dynamic effect of fat and sugar is so much greater that when the effect of sugar alone is subtracted, there remains quite a hump of extra dynamic effect on the fat curve (marked 'difference'). This particular curve is selected for illustration because the subject (V) was probably the most completely reposed subject

who has ever been in the calorimeter, and he was equally so on the cot for Tissot-Haldane determinations. A possible explanation of this phenomenon that can be offered is along the line of the observations by Woods and Merrill ('00) that protein of bread shows a higher coefficient of digestibility when eaten with milk than when eaten alone. Hosoi, Alvarez and Mann ('28) found the same applied to egg white and milk protein. There is ordinarily no interference of fat in the diet with the absorption of other foods (Langworthy and Holmes, '15). While in these observations no systematic controls have been made by ingestion of water alone at a corresponding period after ingestion of fat, it has been observed several times that ingestion of water between periods in order to relieve gastric distress, did not seem to affect the dynamic curve. At all events it would be very surprising if absorption or metabolism of fat would be accelerated to the extent of 8 Cal. per hour by a drink of 300 cc. of water. A still better explanation would seem to be the aid to better oxidation of fat which sugar is known to provide (Shaffer, '21 a, b, c). The next paper will give the data regarding concurrent reduction of ketone bodies. This is the first observation on record, so far as can be discovered, that such a reduction is accompanied by increased heat production.

S.D.A. OF SUGAR WITHOUT EVIDENCE OF COMBUSTION

The object of giving sugar was to test its combustibility at two different intervals after the fat meal. This test is connected with the possible formation of glycogen from fat as developed in an earlier paper (Hawley, Johnson and Murlin, '33). The work is not yet complete and will be reported at a later date.

The two intervals chosen for most of the experiments were 3 to 5 hours after the fat meal, called hereafter 'early' and 11 to 15 hours after, called hereafter 'late,' i.e., at a time when the dynamic action of fat may be supposed to have passed off (fig. 2). With the last three subjects the intervals after fat for giving the sugar were approximately the same. This

was accomplished by giving an extra meal of fat very early in the morning (tables 5, 6 and 7) and following with sugar without an intervening basal. The same amount of sugar was given at the two feedings. In the case of subject VII-'35 (table 4) two doses of sucrose were, for a special purpose, given in the morning, one at 3.05 A.M. and the other at 9.55. Basals were begun at 5 hours and 40 minutes after the first sugar. In spite of these two doses the R.Q.'s following the second showed only a slight rise, proving more conclusively than in any other experiment that the sugar was used chiefly to replenish glycogen. The intervals after sugar ingestion at which the respiratory metabolism was determined by the Tissot-Haldane method were purposely kept about the same (see tables 1 to 7, except 3) for the afternoon, following fat in the morning, and for the early morning experiments.

The work of Mason ('25, '26) and of Deuel ('27) is confirmed by this study in proving that the S.D.A. of carbohydrate cannot be explained by the plethora theory of Lusk. With characteristic frankness, Lusk ('31 a, b) admitted this in his last reviews of the subject. The dynamic rise caused by sugar without any increased combustion of sugar (indeed a fall of a few carbohydrate Calories per hour) is most clearly shown for subject VII-'36 in table 5, and for subject VI in figure 5; but it is also shown for subject I in figure 1, and with only a very slightly increased combustion (only 2 carbohydrate Cal. per hour) in figure 4. Even with subject IV (table 2) who shows an average rise of non-protein R.Q. of 0.08 (from 0.64 to 0.72) the dynamic effect when sugar was fed 'early' after fat produced only 4.3 carbohydrate Cal. per hour, although the total dynamic rise due to sugar was 6.4 Cal. per hour. The next day when fat was not being absorbed the same ingestion of sugar produced a similar average rise of non-protein R.Q. (0.706 to 0.801) and resulted in the combustion of much more sugar—an increase of 23.3 Cal. per hour. With subjects II and VII-'35 the dynamic effect of the sugar was identical whether fed 'early' or 'late' (figs. 3 and 6). The increased combustion of sugar which entered into this effect,

however, was very different in the two subjects. Subject II increased his carbohydrate Calories 30.6 and 33.0 per hour, respectively, in the two conditions, the only one to come so near an equality. Subject VII-'35 experienced an increase in R.Q. from sugar over four times as great in the 'early' state as in the 'late,' notwithstanding an extra 25 gm. sucrose in the very early morning of the 'late' experiment.

The last three subjects afford additional evidence. The second dose of sugar with these subjects followed last fat at the same interval as the first, and yet it sustained a higher combined dynamic effect with no greater increase of non-protein respiratory quotients (tables 5, 6 and 7). Making allowance for better tolerance of fat with developing adaptation, there is no reason to suppose the sugar was not equally well absorbed. Clearly, therefore, there is no proportionality between the specific dynamic effect of sugar and the amount of sugar burned.

SUMMARY AND CONCLUSIONS

1. The specific dynamic action of butter fat, eaten as heavy cream, was studied in nine human subjects by means of the new semi-automatic respiration calorimeter, supplemented by the Tissot-Haldane method. Amounts of fat contained in the test meal varied from 80 to 171 gm. S.D.A. experiments occurred on the fourth or fifth day of an all-cream diet.

2. For non-protein respiratory quotients less than 0.707, heat values for oxygen below the Zuntz-Shumburg table were found by the formula $\frac{4.686 \times \text{low R.Q.}}{0.707}$. The method assumes that all of the non-protein CO_2 under these conditions comes from combustion of fat and that all of the extra oxygen is used for some other purpose than immediate combustion. Direct and indirect calorimetry agreed remarkably well, thus tending to confirm the assumption.

3. Plausible S.D.A. curves showing the entire course of extra heat production over basal were possible for all subjects but one. The average S.D.A. expressed as a percentage of the fat calories fed was 4.74. Not more than 6% of the calory

content was from other foodstuffs. The figures agree fairly well with those of Murlin and Lusk ('15) on the dog.

4. There is no relation of S.D.A. to age, for lowest effects were obtained from the youngest and oldest subjects. It is, however, related to tolerance for fat, meaning not only capacity to digest but also capacity to metabolize completely.

5. With six subjects sugar (sucrose or glucose C.P.[7]) was superimposed on the high fat at an 'early' interval (3 to 5 hours) after the fat meal was taken and the same amount again at a 'late' interval (11 to 15 hours), for the purpose of testing its combustibility. The dynamic response to the sugar was of two types, a) complete summation to the fat metabolism, and b) an increase greater than that due to the same amount of sugar fed alone; the difference is reckoned as fat metabolism. The extra heat may come in part from oxidation of ketone bodies induced by the sugar (see following paper). With three subjects the sugar, glucose, fructose and sucrose, respectively, was given twice at equal intervals after last fat and the combustibility and dynamic effects compared.

6. There is no doubt from these experiments that a strong dynamic effect from sugar may be obtained without any evidence of its own combustion; where such evidence (higher R.Q.) was obtained there was no proportionality to the dynamic effect.

7. The S.D.A. of fat does not appear to be due entirely to plethora in the sense of Lusk, for a) it does not run parallel to blood fat, and b) sugar superimposed causes increased combustion of fat.

[7] Supplied by Corn Industries Research Foundation.

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THE RATE OF KETOGENESIS IN HUMAN SUBJECTS ON HIGH FAT DIETS, AS INFLUENCED BY DIFFERENT SUGARS

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INTRODUCTION

The important work of Shaffer ('21, '22) and of Woodyatt ('21) indicated a close quantitative relationship of ketogenic to antiketogenic factors in the mixture of foodstuffs being oxidized in the body at the threshold of ketonuria. It is as true now, as it was 15 years ago, that the practical management of ketosis is best guided by these quantitative relations. The threshold of ketonuria has been found to occur at or near the K:A ratio of Shaffer or the FA:G ratio of Woodyatt by several different observers (Hubbard and Wright, '22; McClellan, Spencer, Falk and DuBois, '28; McClellan and DuBois, '30). Beyond the threshold, however, important exceptions in yield of ketones in the urine, as compared with the theoretical yield according to Shaffer, have been encountered, especially in obese and other fat-adapted subjects (McClellan, Spencer, Falk and DuBois, '28; Hawley, Johnson and Murlin, '33); in persons living on an exclusive meat diet (McClellan and DuBois, '30), and very recently in depancreatized dogs whose metabolism was increased by exercise and by dinitrophenol (Barker, '36). A further examination of the question of ketone yields in comparison with combustion of carbohydrate, from the standpoint of changes occurring over short periods of time, and employing a new manner of expressing the ketogenesis, would seem to be in order.

[1] Fellow under grant from Corn Products Research Foundation.

In an earlier paper from this laboratory, it was shown that no relation seemed to exist between the level of the R.Q. in persons living on an all cream diet (Hawley, Johnson and Murlin, '33) and the degree of ketosis as measured either by ketonuria or ketonemia. In another communication (Clarke and Murlin, '36) ketolytic effects without evidence of combustion of carbohydrate have been noted in depancreatized dogs and in still another (Murlin and Manly, '36) where the effect of different sugars on ketosis in 4-hour periods, was studied, the greatest reduction was not obtained by the most combustible sugar. In this latter paper a method suggested by Manly of expressing the effect of sugar by means of the change in 'ketone substance production' was adopted. This term is synonymous with the term 'rate of ketogenesis' as used in the present paper. It takes into account both the ketonemia, or level of ketone bodies in the blood, and the ketonuria, or the rate of ketone body excretion in the urine. The amount of ketone bodies excreted, expressed in mg./hr. is increased or decreased, as the case may be, by the change in ketone level multiplied by blood volume, expressed again in mg./hr., according to whether there has been an accumulation of ketones in the circulation or a loss therefrom. The literature bearing on the effects of different sugars on ketosis has been sufficiently reviewed in the two papers just cited.

That aspect of the quantitative relationship of ketolytic to ketogenic factors which has interested us especially in the present study on human subjects, is the relation of carbohydrate combustion to the reduction of ketogenesis. If the body depends upon a stoichiometric combustion of sugar with fatty acid to prevent ketosis, the cure of an already established ketogenesis by feeding sugar should show some evidence of combustion of the sugar.

The relation of ketogenesis to glycogen formation also is important. Concerning this question, it may be pointed out that much new evidence points to the liver as the principal site of ketogenesis (Burn and Ling, '28; Cannavó, '31; Leites and Odinov, '35; Beazell, '35; Edson, '35 a and b; Mirsky,

'36 a and b) as well as the site of other transformations of fat (Gemmill and Holmes, '35; Haarmann, '35); it might be expected, therefore, that the effects of sugar could be explained principally in terms of what happens in the liver. Since this cannot be determined directly in the human subject, one is compelled to rely upon the respiratory metabolism and changes in the blood and urine, which are closely related to glycogen supply and combustion of carbohydrate. It is generally agreed that if the combustion of a dose of sugar follows promptly after its administration, the glycogen level of the liver is relatively high, or at least that the liver is not being hindered in its formation of glycogen. This is one inference which may fairly be drawn from the alteration of tolerance curves (du Vigneaud and Karr, '25) and of the R.Q.'s (Dann and Chambers, '30) with repeated doses of sugar administered to fasting animals. The bearing of the experiments to be described upon the glycogen level as measured by the combustibility of sugars will be discussed.

The paper immediately preceding this one contains the effects on heat production of superimposing sugar at two different intervals after a meal of fat. The primary object of thus spacing the meals of sugar was to test its relative combustibility at two stages of the fat metabolism, one (early) 3 to 5 hours, after fat when ketosis was highest and the other (late) 11 to 15 hours after, when ketosis was definitely on the decline and the specific dynamic action of fat entirely had disappeared. There were considerations with reference to the glycogen storage in the liver also which led to this manner of feeding, but this phase of the investigation has not been completed and will be reported upon at a later date.

Would the effect upon ketogenesis of the two feedings of sugar be proportional to or in any other way ascribable to the increased combustion of sugar? If not, it would appear that a revision of current ideas regarding the prevention of ketosis is necessary. The results from the described spacing of sugar meals have been controlled also by feeding equal doses at equal intervals after fat.

METHOD

Along with the calorimetric work for determination of the S.D.A. of fat (see preceding paper) urine was collected in the appropriate intervals for calculation of the non-protein R.Q. The same collections were used for determination of the ketone bodies. In all but the last three subjects the Hubbard ('21) method was used following oxidation of interfering substances with sodium peroxide and oxidation of β-hydroxybutyric acid to acetone by potassium dichromate. In the last three subjects Van Slyke's ('17) method was used for the total ketones.

Blood samples were taken also, usually just before the urine was collected so that blood intervals and urine periods practically were identical. In six subjects the Shaffer-Hubbard distillations as slightly modified by Behre and Benedict ('26) was used for the ketone bodies of the blood. Instead of the colorimetric determination, however, the Hubbard idometric determination was used. With the last three subjects the Van Slyke and Fitz ('19) method was employed for the blood filtrates. The recovery of pure β-hydroxybutyric acid added to urine and to blood filtrates in both methods averaged close to 75%. Correction has been made on this basis. With the last three subjects also the CO_2 combining power of blood plasma, according to the method of Van Slyke and Neill ('24) was determined.

Only eight of the nine different subjects used in the preceding study furnished bloods for the present study of ketogenesis, but subject VII was used twice. Six of them took the supplementary feeding of sugar from 3½ to 5 hours after the test meal of cream and again the following morning, 11 to 15 hours after further ingestion of cream in the evening. The last three subjects took equal doses of the same sugar at the same intervals after feeding fat both days. Four subjects took sucrose, four C.P. corn glucose and one C.P. fructose (Pfanstiehl).

The procedure with reference to administration of fat meals, the calorimetric determinations and respiratory metabolism,

to which the subject of this paper is so intimately related, have been described fully in the preceding paper. It is necessary to refer the reader to this paper for further details.

RESULTS

Tables 1 to 4 illustrate the fundamental data. Each table concerns one subject, whose roman number is the same as in the preceding paper. Because of limitation of space it is not possible to publish all of the tables. Both in blood and urine acetone and aceto-acetic acid, where this fraction was determined separately, is expressed as acetone, β-hydroxybutyric acid as such and total acetone after conversion of β-hydroxybutyric to acetone. With the Van Slyke and the Van Slyke and Fitz methods only total acetone is reported.

The average non-protein R.Q.'s for the period represented by the urine collection also are given. Blood sugar for subjects I, II, IV and VIII were taken only in relation to the sugar ingestion, but for the remaining five experiments for all blood collections.

The usual increase in ketonemia on the all-cream diet may be noted in tables 1 and 2 from the rise in total acetone from the basal period of the third day to that of the fourth day. Only one exception occurred (subject V), and there is no known explanation. The increase occurs in the β-hydroxybutyric acid in four of the six subjects[2] and in the combined acetone and aceto-acetic in five out of six. With subject V the latter increase is very small. The average increase in total acetone for the six subjects is 8.8 mg.%, which is 33% of the earlier level.

The increase in ketonuria (total acetone) in the basal urine from the third to the fourth day of the same subjects is much greater in absolute figures, but not much greater percentage-wise; namely 141 mg. per hour, which is 41% of the original level (third day).

A single meal of cream given soon after the basal blood on the fourth day of the diet (fifth day for subject IX) and

[2] In table 4 only total acetones are reported.

TABLE 1

Effect of glucose on ketosis and ketonuria (subject IV)

DATE 1934	HOUR	IN BLOOD, MILLIGRAMS PER 100 CC.				AVERAGE NON-PROTEIN R.Q.	IN URINE, MILLIGRAMS PER HOUR			
		Acetone and diacetic as acetone	β-oxybutyric acid	Total as acetone	Sugar		Period	Acetone and diacetic as acetone	β-oxybutyric acid	Total as acetone
January										
30[1]	9.29 A.M. (basal)	15.8	32.3	33.8		0.67	8.04– 9.24 (basal)	8.3	14.5	16.4
	In calorimeter 2 hours—no food									
	12.23 P.M.	36.4	43.4	60.6		0.7	9.24–12.04	22.7	33.3	41.3
	6.30 P.M.	Heavy meal cream + lettuce								
31	9.41 (basal)	25.9	52.7	55.3		0.68	8.11– 9.24 (basal)	61.5	364.3	264.8
	10.05	250 gm. 4X cream (95 gm. fat)—in calorimeter 3 hours								
	1.00 P.M.	29.0	65.6	65.6	56	0.64	9.24– 1.04	88.3	569.8	421.3
	1.30 P.M.	100 gm. glucose in 300 cc. water								
	4.08 P.M.	29.0	43.4	53.2	104	0.72	1.04– 4.11	111.0	504.0	392.0
	6.30 P.M.	250 gm. 4X cream only								
February										
1	9.25 (basal)	25.9	80.4	70.8	80	0.71	8.05– 9.28 (basal)	13.8	22.2	26.2
	9.45 A.M.	100 gm. glucose in 300 cc. water								
	12.27 P.M.	23.6	32.3	41.0	85	0.80	9.28–12.28	9.7	23.9	23.0

[1] Third day of all-cream diet.

TABLE 2

Effect of sucrose on ketosis and ketonuria (subject VI)

DATE MAY 1935	HOUR	IN BLOOD, MILLIGRAMS PER 100 CC.				AVERAGE NON-PROTEIN R.Q.	IN URINE, MILLIGRAMS PER HOUR			
		Acetone and diacetic as acetone	β-oxybutyric acid	Total as acetone	Sugar		Period	Acetone and diacetic as acetone	β-oxybutyric acid	Total as acetone
28[1]	9.40 A.M. (basal)	9.2	16.3	18.3	109	0.73	7.17– 9.33 A.M. (basal)	27.5	52.3	56.5
	In calorimeter 3 hours—no food—no R.Q.'s									
	2.00 P.M.	8.3	16.6	17.6	100		9.33– 2.04 P.M.	28.4	22.1	30.7
	3.00 P.M.	350 gm. 4X cream + lettuce. 6.30 250 gm. 4X cream + lettuce								
29	9.48 A.M. (basal)	11.6	16.6	20.9	120	0.73	7.35– 9.33 A.M. (basal)	26.9	79.6	71.3
	10.10 A.M.	400 gm. 4X cream (152 gm. fat) + lettuce. In calorimeter 4 hours								
	3.06 P.M.	11.1	18.6	21.5	120	0.75	10.20– 3.03 P.M.	57.3	147.0	139.0
	3.10 P.M.	100 gm. sucrose + 300 cc. water								
	6.35 P.M.	4.6	9.4	9.8	215	0.71	3.03– 6.20 P.M.[2]	41.0	111.8	103.0
	7.00 P.M.	200 gm. 4X cream + lettuce								
30	9.30 A.M. (basal)	4.3	8.9	9.3	126	0.75	7.49– 9.33 A.M. (basal)	11.0	12.8	18.0
	9.35 A.M.	100 gm. sucrose + 300 cc. water								
	12.50 P.M.	2.9	7.4	7.0	137	0.86	9.33–12.44 P.M.[3]	6.0	32.8	24.0

[1] Third day of all-cream diet.

[2] 2.45 gm. glucose in urine.

[3] 2.10 gm. glucose in urine.

varying from 240 to 450 gm. raised the ketonemia within an average period of 4.1 hours about 22%; the average increased excretion in milligrams per hour in the corresponding period is 29%. It is evident that neither the rise in blood ketones nor the ketones in the urine alone can express the full effect of the fat meal. Hence it is important to find a better method of expressing the effect not only of a fat meal but also of ketolytic substances to offset the ketogenic. But before preceeding to this matter the alteration in levels of blood ketones

TABLE 3

Effect of fructose on ketosis and ketonuria (subject VIII)

DATE JUNE 1936	HOUR	IN BLOOD, MILLIGRAMS/100 CC.		CO$_2$ COMBINING POWER	AVERAGE NON-PROTEIN R.Q.	IN URINE, MILLIGRAMS PER HOUR			TOTAL KETOSIS	KETOGENESIS
		Total acetone	Sugar			Hour	Nitrogen	Total acetone		
				vol. %					mg.	mg./hr.
1[1]	9.35 A.M.	36.4		43.5	0.75	9.37	504	176	2360	
		In calorimeter, basal, nude								
	1.30 P.M.	37.0		44.5	0.70	1.20	550	302	2400	312.1
	1.40 P.M.	330 gm. 4X cream (132 gm. fat)								
	2.00 P.M.	In calorimeter, nude								
	6.14 P.M.	42.0	67	39.0	0.72	6.02	501	422	2720	490.0
	6.15 P.M.	50 gm. fructose in 300 cc. water								
	9.55 P.M.	35.4	89	38.5	0.95	9.58	442	205	2290	93
2	9.00 A.M.	330 gm. 4X cream (132 gm. fat)								
	9.10 A.M.	41.0	100	38.2	1.02	9.02	268	151	2640	
	9.18	50 gm. fructose in 300 cc. water								
	12.45	32.0	85	44.0	1.21	12.43	283	101	2090	—47

[1] Third day of all-cream diet.

and of their excretion produced by sucrose and glucose may be discussed.

The first or 'early' administration of sucrose came at an interval of 3.5 hours approximately after fat for the first three experiments, at 5 hours approximately for the second three, the difference being explained by the longer calorimetric period in the second group, and 4½ hours for the last three. The next blood sample was taken at 3 hours approximately (average 3.3 hours) from the time of drinking the sugar solution. The average effect of the sugar on blood ketones (total

TABLE 4

Effect of sucrose on ketosis and ketogenesis (subject IX)

DATE 1936 JUNE	HOUR	IN BLOOD, MILLIGRAMS/100 CC.		CO_2 COMBINING POWER	AVERAGE NON-PROTEIN R.Q.	HOUR	IN URINE, MILLIGRAMS PER HOUR		TOTAL KETOSIS	KETOGENESIS
		Ketone bodies as acetone	*Sugar*				Nitrogen	Ketone substance as acetone		
				vol. %					*mg.*	*mg./hr.*
21[1]	9.32 A.M.	8.7	73	52.8	0.71	9.35 A.M.	845	32	588	
	9.42 A.M.	300 gm. 4X cream (123 gm. fat)								
	12.57 P.M.	14.2	66	54.5	0.72	1.00 P.M.	860	80	955	186
22	9.20 A.M.	9.7	67	58.0	0.71	9.22 A.M.	773	25	654	
		In calorimeter 3 hours basal								
	1.15 P.M.	10.6	62	....[2]	0.72	1.16 P.M.	756	52	717	69
	1.22	300 gm. 4X cream (123 gm. fat)								
	6.35 P.M.	18.3	58	45.0	0.70	6.36 P.M.	721	63	1240	232
23	9.29 A.M.	25.8	70	56.0	0.72	9.30 A.M.	634	210	1745	....
	9.47 A.M.	300 gm. 4X cream (123 gm. fat)								
		In calorimeter 4 hours								
	2.46 P.M.	34.5	64	50.0	0.69	2.48 P.M.	631	495	2330	637
	2.50 P.M.	50 gm. sucrose in 300 cc. water								
	6.05	12.9	117	56.0	0.70	6.06 P.M.	595	249	873	— 192
24	4.55 A.M.	300 gm. 4X cream (123 gm. fat)								
	9.57 A.M.	36.1	76	58.0	0.73	10.00 A.M.	417	347	2440	
	10.01 A.M.	50 gm. sucrose in 300 cc. water								
	1.24 P.M.	10.3	91	63.0	0.70	1.25 P.M.	426	144	697	— 383

[1] Third day of all-cream diet.

[2] Sample lost.

as acetone) was a reduction of 8.3 mg. per 100 cc. The range is quite wide; namely, from an elevation of 4.9 mg.% after 50 gm. glucose, to a fall of 21.6 mg.% after 100 gm. sucrose (table 5). The average percentage reduction with sucrose was 38; with glucose 11. Percentage reduction, however, is not a satisfactory basis of comparison, for the reason that the group who took glucose happened to contain two of the subjects with lesser tolerance (see preceding paper), while the group taking sucrose contained only one such individual.

TABLE 5

Comparative effects of sugars on ketonemia

SUBJECT NO.	AMOUNT	SUGAR	ORIGINAL LEVEL	Change in 3 hours
		First dose		
	gm.		mg. %	mg. %
VII-'35	25	Sucrose	33.3	— 6.7
VII-'36	25	Glucose	19.3	+ 1.9
IX	50	Sucrose	34.5	— 21.6
I	50	Glucose	41.4	+ 4.9
VIII	50	Fructose	42.0	— 6.6
IV	100	Glucose	65.6	— 12.4
II	100	Sucrose	48.4	— 6.8
V	100	Glucose	33.0	— 16.0
VI	100	Sucrose	21.5	— 11.7
Average		Sucrose	34.4	— 10.7
Average		Glucose	39.8	— 5.4
		Second dose (18 hours later)		
VII-'36	25	Glucose	33.2	— 4.9
VIII	50	Fructose	41.0	— 9.0
IX	50	Sucrose	36.1	— 25.8

The original level of the ketones therefore, when the glucose began to exert its effect, was higher, resulting in lower percentage reductions. Average absolute reduction in milligram per cent is patently the fairer method of comparison. On this basis the reduction of total acetone was 10.7 mg% for sucrose and 5.4 mg.% for glucose. There is no close relationship apparent between size of dose and the effect. Twenty-five grams sucrose in one individual produced as great an effect as

100 gm. in another, and 50 gm. produced a greater effect (subject IX). Fifty grams glucose in one individual produced more than twice as great a rise as 25 gm. in another, while 100 gm. produced approximately the same fall in two others. Wierzuchowski ('25) noted that in phlorhizinized dogs the original level of ketosis seemed to determine the extent of the fall produced by any given dose of glucose (the higher the level the greater the fall) and this is borne out by experiments reported from this laboratory (Murlin and Manly, '36). But the present data on normal humans do not exhibit this phenomenon. The comparisons except in one instance are made between different individuals. It seems impossible to make a comparison of two different doses, whether of the same or different sugars on the same individual, without having a hang-over effect of the first dose on the second unless a very considerable period of time has elapsed between the two tests.

The effect of a second dose of the same sugar on the same individual repeated at the same interval after last fat as the first, so as to secure if possible the same initial level, of ketonemia, is exhibited in the lower part of table 5. The second dose followed the first at an average interval of 18 hours (20, 15 and 19). Without exception the second dose produced a greater effect than the first and in two instances the initial levels were very nearly the same. However, the differences are not great, indicating that if the effect of fat had been permitted to hold sway in those subjects for a few hours longer, the hang-over effect would have disappeared.

It is quite clear from the data presented that sucrose is considerably more effective in reducing ketonemia in 3 hours than is glucose.

EFFECT ON KETONURIA

When we assemble the effects on different subjects side by side, as in table 6, it is seen again that there is no proportionality between size of dose and extent of the effect. Twenty-five grams sucrose raised the excretion rate slightly while 50 gm. lowered it considerably and 100 gm. reduced it less.

Twenty-five grams glucose increased the excretion rate approximately one-half as much as 50 gm., while 100 gm. reduced it a little in one case and raised it a little in the other. A high initial level of excretion seems to make the rate more susceptible of reduction than a lower level.

TABLE 6

Comparative effects of sugar on ketonuria

SUBJECT NO.	AMOUNT	SUGAR	KETONURIA, MILLIGRAMS PER HOUR	
			Initial level	CHANGE IN 3 HOURS
First dose				
VII-'35	*gm.* 25	Sucrose	120	+ 20
VII-'36	25	Glucose	56	+ 141
IX	50	Sucrose	495	— 246
I	50	Glucose	159	+ 301
VIII	50	Fructose	422	— 217
II	100	Sucrose	273	— 90
IV	100	Glucose	421	— 29
VI	100	Sucrose	139	— 36
V	100	Glucose	224	+ 12
Second dose (18 hours later)				
VII-'36	25	Glucose	697	+ 168
VIII	50	Fructose	151	— 50
IX	50	Sucrose	347	— 203

COMPARATIVE COMBUSTIBILITY OF SUGARS

When the data for changes in blood sugar, in average non-protein respiratory quotients, in level of blood ketones and total ketosis (see below) are brought together, the results are as shown in table 7. The changes for blood sugar and ketonemia are calculated from determinations made just before the first dose of sugar and at 3 hours (average 3.3 hours) following its ingestion. The respiratory metabolism periods from which change in non-protein quotients are calculated, in all cases, immediately preceded taking of blood and urine samples, so that they fairly indicate the mixture of non-protein foodstuffs being oxidized over 3 or more hours leading up to the blood conditions found. Fat in large quantity was present

in the alimentary tract (see tables 1 to 4) while the first quotients were being obtained and fat plus sugar for the later ones.

A graphic method is not required to show the absence of parallelism either between blood sugar change and change in ketonemia or between change in the character of combustion and effect on ketosis. In all cases but one (VII-'36) blood sugar was accumulating during the 3 hours following the

TABLE 7

Combustibility of sugars in relation to ketosis

SUBJECT NO.	AMOUNT	SUGAR	CHANGE IN LEVEL BL. SUGAR	CHANGE IN CO_2 COMBINING POWER	CHANGE IN KETONEMIA	CHANGE IN NON-PROTEIN R.Q.	CHANGE IN TOTAL KETOSIS
First dose							
	gm.		*mg. %*	*vol. %*	*mg. %*		*gm.*
VII-'35	25	Sucrose	+ 5		— 6.7	+ 0.10	— 0.53
VII-'36	25	Glucose	— 35	— 4.4	+ 1.9	— 0.02	+ 0.16
IX	50	Sucrose	+ 53	+ 6.0	— 21.6	+ 0.01	— 1.37
I	50	Glucose	+ 54		+ 4.9	— 0.01	+ 0.42
VIII	50	Fructose	+ 22	— 0.5	— 6.6	+ 0.23[1]	— 0.41
II	100	Sucrose	+ 105		— 6.8	+ 0.12	— 0.45
IV	100	Glucose	+ 48		— 12.4	+ 0.08	— 0.96
VI	100	Sucrose	+ 95		— 11.7	— 0.04	— 0.72
V	100	Glucose	+ 32		— 16.0	+ 0.01[1]	— 1.03
Second dose (18 hours later)							
VII-'36	25	Glucose	+ 13	— 3.7	— 4.9	+ 0.01	
VIII	50	Fructose	— 15	+ 5.8	— 9.0	+ 0.199[1]	
IX	59	Sucrose	+ 15	+ 5.0	— 25.8	— 0.03	

[1] This subject exhibited hyperpnoea.

ingestion, proving that absorption was going on rapidly. The peak of the absorption curve naturally must have come before the 3.3 hours (average) collection of blood. The figures show not the extent of absorption, but rather the amount of sugar remaining in circulation at this time. The level remaining is not proportional to the amount given but reflects rather the tolerance, meaning capacity for removal of blood sugar. In four cases (VII-'35, VIII,[3] II, IV) the rise in R.Q. proves

[3] This subject only exhibited definite hyperpnoea probably accounting in some measure for the rise in R.Q., though it was present in both series of respiratory determinations.

that combustion of sugar must have been taking place tending to reduce the blood sugar. In all these tests (two after sucrose, one after fructose and one after glucose) the ketonemia at the same time was falling. The rise in R.Q. therefore was not influenced by greater ketosis and consequently greater release of CO_2 through the lungs (see discussion of CO_2 combining power below). In only one other case (VI) is the change in R.Q. significant and in this instance it fell. Without significant change in carbohydrate combustion, as the remaining subjects show, ketosis may rise slightly or fall considerably under the influence of sugar. The only other way of disposing of carbohydrate (aside from excretion, which never amounted to more than 1 or 2 gm., or fat formation which could scarcely take place without a significant rise in R.Q.) is glycogen formation. Including effects of the second dose of sugar (bottom of table 7) there are five instances of definite reduction of ketonemia within 3.3 hours without evidence of concomitant combustion of sugar. Three of these occur following sucrose and two following glucose. There are two instances of slight increase in ketonemia without evidence of combustion. If we assume that most of the effect to reduce ketonemia occurs in the liver (p. 647) where ketogenesis itself is believed mainly to take place, perhaps the last two cases, both with smaller doses of glucose, may be explained as a preliminary displacement of ketone substances from the liver.

It is unfortunate that the CO_2 combining power of the blood plasma was not determined in all the experiments. In three of the instances where it was determined a definite rise in this property coincided with a considerable reduction of the ketonemia, as would be expected. In two instances where the ketonemia changed but little (a rise of 1.9 and a fall of 4.9 mg.%) there was a concomitant small decrease in combining power, one accompanied by a fall of blood sugar, the other by a small rise. With at least two others showing decrease in ketonemia and increase in respiratory quotient, it seems probable that a rise in CO_2-combining power must have occurred even though it was not determined.

The last column of table 7 contains the term 'total ketosis.' By this term is meant the total amount of ketone substances expressed as acetone contained in the entire circulation. This value is found by multiplying the blood volume taken as 8.8% of the body weight in kilograms (Keith, Rowntree and Geraghty, '15; Seyderhelm and Lampe, '25) by the ketonemia in milligrams per cent. The change in total ketosis naturally bears the same sign as the change in milligrams per cent. But in comparing different subjects the two values are not in the same proportion on account of differences in weight. Change in total ketosis, however, bears no closer relationship to changes in combustion or to changes of blood sugar levels.

Concluding this section, it may be stated emphatically that reduction of ketosis does not appear to depend upon combustion of sugar.

RATE OF KETOGENESIS AND CARBOHYDRATE COMBUSTION

The concept of rate of ketogenesis has been sufficiently explained (p. 646). It was devised as an expression of total ketone production as measured by change in total ketosis and in ketonuria in relation to time. A positive value may mean that ketone substances are being accumulated in the body in addition to being excreted, or falling less rapidly in the body than being excreted. A negative value means decrease in the body more rapidly than they are being excreted. Ketone substances then are being disposed of in some other way (presumably by oxidation).

The data for comparison of change of rate in ketogenesis with changes of carbohydrate combustion are shown in table 8. Sucrose invariably caused a decreased rate. Glucose in small quantity (25- and 50-gm. doses) caused an increased rate; but in larger quantity (100 gm.) caused twice as great a decreased rate as the same quantity of sucrose. All these effects were measured to 3 hours (average 3.3 hours) after ingestion. Prolonged over a greater period (8 hours) glucose reduces ketogenesis in the phlorhizinized dog (Murlin and Manly, '36) more than sucrose or fructose. Quite probably this would

be true also in these human subjects, for in three of them who took glucose the ketonuria fell much farther over night between the fourth and fifth days of the diet, than it did in three who took sucrose. The ketonemia fell less for glucose. The ingestions of fat, however, were not equalized and the collections of blood and urine were not carried out in such a

TABLE 8

Comparison of rate of ketogenesis with carbohydrate combustion

SUBJECT	FAT OR SUGAR	RATE OF KETOGENESIS	CHANGE IN RATE OF KETOGENESIS	CHANGE IN NON-PROTEIN R.Q.	CHANGE IN CARBOHYDRATE METABOLISM
		mg./hr.	*mg./hr.*		*Cal./hr.*
VII-'35	152 gm. fat	148			
	25 gm. sucrose	— 20	— 168	+ 0.10	+ 26.0
VII-'36	140 gm. fat	171			
	25 gm. glucose	245	+ 74	— 0.02	— 2.5
IX	123 gm. fat	637			
	50 gm. sucrose	— 192	— 829	+ 0.01	— 0.35
I	171 gm. fat	337			
	50 gm. glucose	617	+ 280	— 0.01	— 1.10
VIII	132 gm. fat	490			
	50 gm. fructose	93	— 397	+ 0.23[1]	+ 44.7[1]
II	91 gm. fat	227			
	100 gm. sucrose	10	— 217	+ 0.12	+ 31.0
IV	95 gm. fat	661			
	100 gm. glucose	83	— 517	+ 0.08	+ 4.3
VI	152 gm. fat	146			
	100 gm. sucrose	— 102	— 248	— 0.04	— 11.0
V	155 gm. fat	366			
	100 gm. glucose	— 67	— 433	+ 0.01	+ 2.8

[1] This rise of R.Q. and carbohydrate calories is questionable because of hyperpnoea; the only subject presenting this complication.

way as to make possible the calculation of the rate of ketogenesis for the night period.

Subjects II, IV, V and VI in this table fall into two paired experiments. The first two were people of comparatively low tolerance for fat; the latter two of comparatively high tolerance (tables 1 and 2). They were fairly well-paired also for weight. Approximately the same meal of butter fat in

each pair was followed at the same interval by sucrose in one member and glucose in the other. The rate of ketogenesis was changed by sucrose, in the two receiving that sugar, to nearly the same extent. The comparison for glucose is not quite so close; but in each pair its effect is unmistakably greater than that of sucrose—mean for the two sugars — 425 and — 232 mg. per hour, respectively.

Change in average R.Q. and in carbohydrate combustion, expressed as Calories per hour, from three periods just preceding and just following the first dose of sugar (tables 1 to 7, preceding paper) are shown in the last columns of table 8. It is evident at a glance that no parallelism between either of these changes and the change in ketogenesis exists. Increase in carbohydrate combustion occurs with decreased ketogenesis in four subjects (excluding VIII) and the reverse occurs in two (VII-'36 and I). Thus far and no farther do the results fit into the theory of Shaffer for ketogenesis-antiketogenesis. The respiratory quotients and the determination of ketone substances were as accurate, we believe, as it is possible to make them. Signs of 'blowing off' CO_2 were present in only one subject.[4]

In detail the quantitative relationship falls down completely. For example, the person (subject IX) exhibiting the greatest reduction of ketogenesis showed only a negligible change of carbohydrate metabolism. Other detailed comparisons in the table show no proportionality between the changes of rate of ketogenesis and antiketogenesis.

EFFECTS OF SUGAR GIVEN 'EARLY' AND 'LATE'

There remain to be compared the effects on the rate of ketogenesis in comparison with the extra carbohydrate combustion caused by the same dose of the same sugar administered 'early' and 'late' after the last fat was taken (table 9).

[4] Subject VIII who exhibited such signs is excluded, although with definite hyperpnoea he fits the theory; and for subject VII-'36, who exhibited hyperpnoea in two periods after fat on June 8th (p. 632 of preceding paper), only the third period was used.

Only subject II exhibits anything like a proportionality between the total ketosis, and the amount of carbohydrate oxidized, and none shows any proportionality between change of ketogenesis and of carbohydrate combustion. Nearly the same extra oxidation per hour following the ingestion of 100 gm. sucrose 'early' and 'late' by subject II accompanied a like reduction in absolute level of ketones in the body. But from the ketogenesis figures it is evident that this result is due to a disappearance of ketones amounting to 142 mg. per

TABLE 9

Comparative effects of sugar given early (E) and late (L) after fat meal

SUBJECT	SUGAR	CHANGE IN TOTAL KETOSIS	CHANGE IN RATE OF KETOGENESIS	CHANGE IN CARBOHYDRATE METABOLISM
		gm.	*mg./hr.*	*Cal./hr.*
I	50 gm. glucose	E + 0.42	+ 617	— 1.1
	50 gm. glucose	L — 0.35	— 46	+ 16.1
II	100 gm. sucrose	E — 0.45	+ 10	+ 30.6
	100 gm. sucrose	L — 0.42	— 142	+ 33.6
IV	100 gm. sucrose	E — 0.96	+ 83	+ 4.3
	100 gm. sucrose	L — 2.31	— 738	+ 23.3
V	100 gm. glucose	E — 1.03	— 67	+ 2.8
	100 gm. glucose	L — 0.21	— 52	+ 30.2
VI	100 gm. glucose	E — 0.72	— 102	— 10.9
	100 gm. glucose	L — 0.14	— 18	+ 28.7
VII	25 gm. sucrose	E — 0.54	— 20	+ 25.8
	25 gm. sucrose	L— 0.49	— 89	+ 5.8

hour following the 'late' dose, as compared with an increased formation of 10 mg. per hour following the 'early' dose. For subject I it can be said that the oxidation of 16 Cal. per hour from carbohydrate accompanied a disappearance of 46 mg. of ketones per hour. This is the effect of late administration; but a slight reduction in carbohydrate calories after the first administration was accompanied by a piling up of 617 mg. per hour. For subject IV slight oxidation 'early' accompanied reduction in level but the excretion proved that there was still a considerable ketogenesis. Five times as much oxidation 'late' accompanied more than twice as much

accumulation, in spite of the disappearance of nearly ¾ of a gram ketones per hour. For the remaining three subjects greater disappearance (negative ketogenesis) goes along with lesser oxidation, while in each case lesser reduction of level goes with greater oxidation.

In every subject except VII oxidation of sugar takes place more readily when the fat is out of the way (11½ to 15 hours after last meal). In three of the five this greater oxidation is accompanied by greater disappearance of ketones; in the other two just the reverse. Including subject VII we have exactly reverse effects in half the subjects. The favorable effects therefore cannot be ascribed to oxidation of sugar. Furthermore, since 50 gm. glucose with subject I produced nearly as much disappearance of ketones (late) as did 100 gm. in subject V; and since 25 gm. sucrose in subject VII produced five times as much disappearance (late) as did 100 gm. of the same sugar in the same time in subject VI (notwithstanding abundant evidence of their absorption into the blood) we must conclude that there is no other stoichiometric relationship between sugar and fatty acids in relation to ketolysis, under these conditions.

DISCUSSION

It would not be profitable at this time to calculate by Shaffer's first ('21 b) or second ('22) method the K:A ratios, because his fat factors are derived from an arbitrary mixture of the higher fatty acids found in human food and the human body, whereas the fat fed in these experiments was of a wholly different composition. Butter fat from cows fed on normal rations according to the most reliable data (Holland, Garvey, Pierce et al., '23) has the following composition of fatty acids, from which the composition of the neutral fat, may be readily calculated (Holland, Reed and Buckley, '15).

	Fatty acid %	*Neutral fat* %
Butyric acid	2.93	3.71
Caproic acid	1.90	2.29
Caprylic acid	0.79	0.90
Capric acid	1.57	1.70
Lauric acid	5.85	6.31
Myristic acid	18.78	21.00
Palmitic acid	15.17	15.89
Stearic acid	14.91	15.44
Oleic acid	31.89	32.85
Total	94.79	100.09

This distribution would give an average neutral fat of the formula $C_{49.3}H_{92.6}O_6$. The triglyceride would have the composition $C_3H_5(C_{15.4}H_{29.2}O_2)_3$ which on complete oxidation would yield an R.Q. of just 0.71 and the FA : G R.Q. i.e., after conversion of all the glycerol to glucose (Shaffer, '21 c), would be 0.688. However, there is no certainty that the nine different fatty acids would be absorbed in the same proportion in which they are present in cream, particularly in a 3- to 5-hour period such as has been considered in this paper, or that they would reach the tissues in this proportion one to another. Such a calculation must wait for more reliable data on differential absorption. Hughes and Wimmer ('35) have shown that the water soluble fatty acids, especially butyric and caproic, must in all probability be absorbed by the portal system and that as a consequence they probably escape the glyceride synthesis. The more rapid passage of these lower fatty acids to the liver naturally would facilitate their transformation to ketone bodies as compared with fatty acids which must travel by the thoracic duct and then be broken down farther to form ketone substances.

A more important matter is the effect of the ketolytic action of sugar on the actual as compared with the theoretical change in respiratory metabolism. Lusk ('28, p. 671) has shown that the formation of 36 gm. β-hydroxybutyric acid from 100 gm. neutral fat would reduce the R.Q. from 0.707 to 0.669. But if this amount of acid were to neutralize $NaHCO_3$, sufficient CO_2 would be released to restore the R.Q. to 0.715. Reversing this

process by combustion of β-hydroxybutyric acid, Na would be released which then would bind up CO_2 causing a reduction of the R.Q. According to the hypothesis of Shaffer ('22), this combustion would require the simultaneous combustion of sugar (glucose or fructose) in the proportion of 1 mol. to 2 of aceto-acetic acid.

The maximum effect of sugar to reduce ketogenesis in this work is shown in table 8, where with subject IX 50 gm. sucrose caused a change of — 0.829 gm. per hour expressed as acetone. Expressed as aceto-acetic acid the amount would be 1.32 gm. per hour. Oxidation of this and the oxidation of ½ mol. of glucose or fructose per mol. of aceto-acetic would produce the following effect on the pre-sugar metabolism of this subject June 23, 1936, table 7, preceding paper).

	CO_2. L./hr.	O_2. L./hr.	R.Q.
Average respiratory exchange before sugar	13.98	19.70	0.71
Oxidation of 1.32 gm. aceto-acetic	1.28	1.28	
	15.26	20.98	0.73
Oxidation of 1.29 gm. glucose	0.96	0.96	
	16.22	21.94	0.74
Subtract CO_2 bound by Na, set free from 1.32 gm. aceto-acetic	0.32		
	15.90	21.94	0.725

The actual net change in R.Q. in this case (table 8), corresponds very closely to the theoretical, but it is the only one which does. It may be objected that the change of R.Q. caused by the elimination of even a maximum ketosis is so small that it might well be obscured in the total respiratory exchange. This can scarcely be an answer to the diverse relations between change of ketogenesis brought about by carbohydrate and the rate of carbohydrate metabolism disclosed by this study. There must be some other function served by carbohydrate in ridding the body of ketone substances. It may be only preparatory to the kindling action.

There is growing evidence that this function is glycogen formation in the liver. Thannhauser ('29) has reviewed it to 1929. His own conviction is contained in the statement, "we see as well in experimental pancreatic diabetes as in phlorhizin diabetes and in severe human diabetes that glycogen impoverishment of the liver and ketonuria are very intimately related" ('29, p. 311). Of the newer evidence mention may be made especially of the work of Steppuhn and Timofejewa ('30) who find a close relationship between the ketosis and the increased protein metabolism of diabetes brought about through activation of proteolytic enzymes in the liver by the acidification of the ketone substances. They picture the sequence of events as follows: depletion of glycogen and mobilization of peripheral fat into the liver and formation of ketones→production of local decrease of pH in the liver→increased activity of proteolytic enzymes and protein destruction formation of glycogen from protein→removal of ketones. "We hold to the fact," they say, "and the whole literature supports the view, that the ketone level is . . . . an excellent glycogenometer of the liver" (loc.cit., p. 471). The recent work of Shapiro ('35) in Deuel's laboratory directs attention to the parallelism between ketolytic action and glycogen forming capacity in rats of glucose, l (+)-lactic acid, pyruvic acid and glycerol. In striking confirmation of the ideas of Steppuhn and Timofejewa was the ketolytic effect of ethyl alcohol, accounted for by an increased catabolism of protein.

SUMMARY

1. In connection with the determination of specific dynamic action of butter fat and of superimposed sugar reported in the preceding paper, the urines collected for nitrogen analysis and blood samples, appropriately spaced, were analyzed for the ketone substances and sugar in eight of the nine subjects. It thus was possible to compare ketogenesis on the high fat diet with the respiratory metabolism and changes induced in both by the administration of sugar (C.P. corn glucose, fructose and sucrose).

2. Analysis, like the calorimetric observations, began on the third day of the all-cream diet. The increase in ketonemia from the third to the fourth day was 8.8 mg.%, or 33% greater. The increase in ketonuria was 41%. A meal of fat containing from 240 to 450 gm. 4X cream given on the fourth day increased the ketonemia in an average period of 4.1 hours 22%; the average increased excretion in the same time was 29%.

3. Sugar was administered at two intervals after the fat, 3 to 5 hours, called 'early' and 11 to 15 hours, called 'late.' Doses varied from 25 to 100 gm. The average effect of the 'early' dose on blood ketones in 3 hours (average 3.3 hours) was a reduction of 8.3 mg.%—the range from + 4.9 mg.% to — 21.6 mg.%. The average reduction with sucrose was 10.7 mg.% and with glucose 5.4 mg.%. There was no definite relation of the effect to the amount of sugar given. Excretion was sometimes reduced and sometimes increased.

4. Blood sugar increased in all cases but at the 3.3 hour sampling varied from — 35 mg.% to + 105 mg.% from the level before sugar ingestion. No parallelism was shown between blood sugar level and change in ketonemia or in total ketosis (meaning mg.% in blood multiplied by blood volume, taken as 8.8% of body weight).

5. Change in rate of ketogenesis, calculated from the total ketosis and the ketonuria, in milligrams per hour varied from + 280 mg. to — 828 mg. Sucrose invariably caused a decrease, while glucose in smaller doses (25 and 50 gm.) caused an increase and in larger doses (100 gm.) a decrease. In two paired experiments (100 gm. of sugar following nearly equal feedings of cream, the two subjects in each comparison being nearly equal in weight) glucose caused a decrease twice as great as sucrose.

6. The increased combustion of carbohydrate in five experiments was more than sufficient to extinguish the ketogenesis according to Shaffer's theory, but in no case did so within 3 to 4 hours. In one experiment out of nine the actual change agreed with theory. In the remaining three there was a decrease in carbohydrate oxidized. The CO_2-combining power

of the blood increased in two experiments with sucrose and one with fructose; it decreased in two with glucose and one with fructose. The R.Q. changes found apparently were not affected by changes in CO_2-combining power except in one instance with fructose.

7. In comparing the 'late' dose of sugar with the 'early' it was found that the change in rate of ketogenesis was negative following the 'late' dose in all of the six experiments, whereas the 'early' dose caused a positive change in three of the six. In all but one experiment there was a greater combustion of sugar following the 'late' dose than following the 'early.'

CONCLUSIONS

1. Sugar causes a marked reduction in total ketosis within 3 to 4 hours.

2. Sucrose is somewhat more effective in doses up to 50 gm.; glucose more effective in 100 gm. doses. Fructose stands between the other two.

3. None of the sugars completely extinguished the ketogenesis in 3 to 4 hours, although the increase in carbohydrate combustion was more than sufficient, according to Shaffer's theory. In one instance out of nine the change in ketogenesis exactly agreed with the increased combustion, according to theory on the basis of 1 mol. glucose to 2 mols, aceto-acetic acid.

4. A 'late' dose of sugar following an 'early' dose was much more effective than the 'early' one in reducing ketogenesis. Also it increased carbohydrate combustion more. This is interpreted as due to replenishment of glycogen in the liver, possibly because of insulin production stimulated by the early dose.

5. Glycogen formation seems to be as important as combustion in the antiketogenic action of sugar. Quite possibly the presence of a certain minimal 'normal' content of glycogen in the liver is a condition precedent to the normal oxidation of fatty acids. There is somewhat more evidence, however, that this normal content of glycogen prevents the formation of ketone substances in the liver.

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THE AVAILABILITY OF *d*(—)-LYSINE FOR GROWTH [1]

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ONE FIGURE

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Several years ago, in a study of the replaceability of lysine in the diet by related hydroxy compounds, McGinty, Lewis and Marvel ('24–'25) noted that natural *l*(+)-lysine promoted somewhat better growth than did synthetic *dl*-lysine. Recently we have verified this observation in an extensive series of tests (Berg and Dalton, '34) which suggested that the unnatural *d*(—)-lysine half of the *dl* mixture could probably not be utilized at all by the rat for growth purposes. For direct proof of this possibility we undertook the resolution of *dl*-lysine (Berg, '36) and are now presenting data obtained on a series of animals fed lysine-deficient diets supplemented with natural *l*(+)-lysine or with its optical *d*(—) antipode.

EXPERIMENTAL

The lysine-deficient diet employed contained zein 19.5, tryptophane 0.2, cystine 0.2, histidine monohydrochloride 0.1, sucrose 15.0, starch 34.5, hydrogenated vegetable oil[2] 19.0, cod liver oil 5.0, salt mixture (Hawk and Oser, '31) 4.5, and agar 2.0%. This was supplemented by feeding two 100 mg. 'vitamin-B complex' tablets (Harris) separately to each animal daily. Zein was used as the chief source of nitrogen because it is apparently entirely devoid of lysine. It was

[1] The designation of generic series by letter and direction of rotation by sign is in accordance with the system of Freudenberg (see Berg, '36).

[2] Crisco.

fed at a higher level than we have usually fed our proteins or protein hydrolysates because it has been shown to escape digestion in part (Mason and Palmer, '34). The use of tryptophane as a supplementing agent is, of course, necessary. To avoid the possibility of cystine or histidine deficiencies, these amino acids also were supplied. Histidine addition was later found unnecessary and was therefore subsequently omitted. The vitamin B complex was used instead of yeast to avoid unnecessary addition of extraneous protein. The zein was prepared by extracting corn gluten with 75% alcohol, precipitating with water, redissolving in 70% alcohol, filtering, concentrating, and reprecipitating with acetone, essentially as directed by Brazier ('30).

The diet was fed to a lot of twenty young rats from four litters for a period of 8 days, after which the animals were separated into seven groups of three each, except one which contained only two. Three of these groups now were given supplements of *d*(—)-lysine and three, supplements of the *l*(+) modification at levels of 0.5, 0.25 and 0.125%, respectively, replacing an equal quantity of zein. The diet of the remaining animals was left unchanged. The lysine was fed as the dihydrochloride; an equivalent amount of sodium bicarbonate was added to the diet to neutralize the hydrochloric acid thus included. The animals were housed in false bottomed cages and received food and distilled water ad libitum. The lysine isomers used were obtained by resolution of *dl*-lysine. The method of their preparation and their melting points, N content, and optical rotations have already been recorded (Berg, '36). After 20 days on the above diets, the histidine monohydrochloride was omitted in all cases, control animals having shown that its inclusion resulted in no growth acceleration. After 80 days, the lysine supplement was omitted from the diets of the animals of the *l*(+) series; the rats fed the *d*(—) supplements and the two remaining animals which had received no supplement were fed 0.5% of *l*(+)-lysine. Twelve days later the experiment was terminated.

DISCUSSION

The results are summarized in figure 1 and table 1. In every case the rats on the *l*(+)-lysine supplements showed definite growth responses. On the other hand, no difference

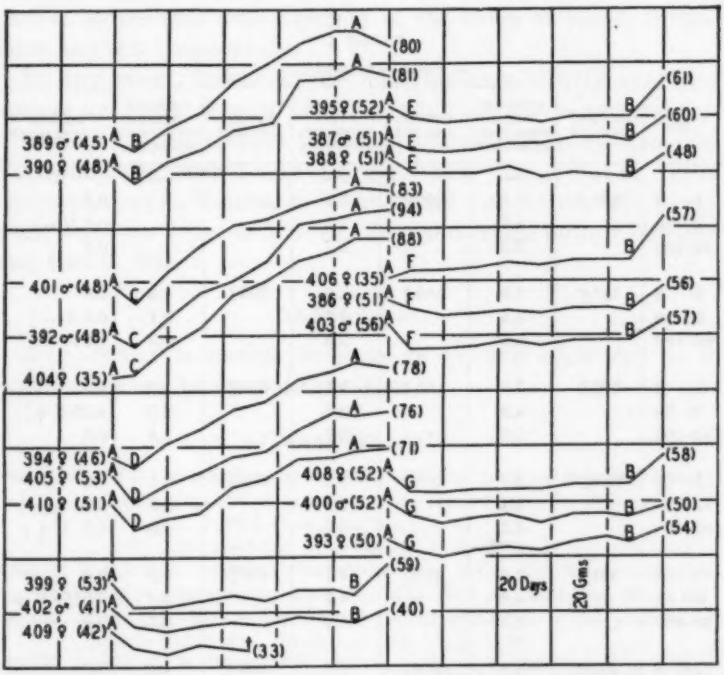

Fig. 1 Growth on *l*(+)- and *d*(—)-lysine. Initial and final weights of the rats are given in parentheses. A represents the unsupplemented lysine-deficient diet. B, C and D indicate similar diets containing 0.5, 0.25 and 0.125% *l*(+)-lysine supplements, respectively; E, F and G, diets containing corresponding supplements, respectively, of *d*(—)-lysine. The dagger indicates the death of rat 409.

was detectable, either in growth or behavior, between the rats fed *d*(—)-lysine and those receiving no lysine supplement. In general, animals which showed a definite growth response also showed a greater food consumption. The preliminary and after periods show that the lack of added *l*(+)-lysine in

the diet either results in loss of weight or permits extremely slow growth; addition of *l*(+)-lysine to the diet of the rats which had previously grown only very slowly, if at all, caused a definite growth response, thus indicating that the 88-day plateau was the result only of an unfavorable dietary regimen.

TABLE 1

Food consumption

DAYS	RAT NO. AND SEX	AVERAGE DAILY FOOD CONSUMPTION	RAT NO. AND SEX	AVERAGE DAILY FOOD CONSUMPTION	RAT NO. AND SEX	AVERAGE DAILY FOOD CONSUMPTION	LYSINE SUPPLEMENT
		gm.		gm.		gm.	%
1– 8	389♂	3.5	390♀	3.3			0.0
9– 88		4.2		4.3			0.5 *l*(+)
89–100		3.3		4.7			0.0
1– 8	387♂	3.8	388♀	2.9	395♀	4.0	0.0
9– 88		3.4		2.5		3.1	0.5 *d*(—)
89–100		3.5		2.3		4.3	0.5 *l*(+)
1– 8	392♂	3.0	401♂	3.1	404♀	3.3	0.0
9– 88		4.0		4.6		4.5	0.25 *l*(+)
89–100		3.0		3.7		3.8	0.0
1– 8	386♀	3.5	403♂	3.3	406♀	3.1	0.0
9– 88		3.4		2.7		2.9	0.25 *d*(—)
89–100		3.2		3.2		4.0	0.5 *l*(+)
1– 8	394♀	3.5	405♀	3.4	410♀	3.0	0.0
9– 88		4.6		4.7		3.7	0.0125 *l*(+)
89–100		3.8		4.1		3.5	0.0
1– 8	393♀	4.0	400♂	3.6	408♀	4.0	0.0
9– 88		3.3		3.0		3.3	0.125 *d*(—)
89–100		3.3		2.9		3.5	0.5 *l*(+)
1– 8	399♀	3.8	402♂	2.5	409♀	3.5	0.0
9– 88		2.9		2.1		2.9[1]	0.0
89–100		3.1		2.0			0.5 *l*(+)

[1] Nine to 50 days.

In the present series of studies the animals on 0.5% and 0.25% *l*(+)-lysine supplements grew at very similar rates; in the earlier studies (Berg and Dalton, '34) the weight increment on the higher lysine supplement was appreciably greater.

Corresponding discrepancies in food consumption in the two series were also observed. The same lot of zein was used in both studies. In the first series it was fed at a 15% level, in the second at 20%. The only other significant difference in regimen was the nature of the vitamin B complex. In the earlier series this was supplied in the form of yeast, in the later as yeast concentrate.

In any event, however, the data indicate clearly that the optical antipode of natural *l*(+)-lysine cannot be utilized for growth.[3] In this respect lysine is comparable to cystine (du Vigneaud, Dorfmann and Loring, '32), but different from tryptophane (du Vigneaud, Sealock and Van Etten, '32; Berg, '34), histidine (Cox and Berg, '34), and methionine (Jackson and Block, '33).

CONCLUSION

d(—)-lysine is unable to promote growth when fed as a supplement in a lysine-deficient diet.

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